Exponent®

Pathogen Sampling and Preliminary Human Health Risk Assessment — Saybrook Place Combined Sewer Overflow, Newark, New Jersey

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Acronyms and Abbreviations

ASI	Analytical Services, Inc.
BGMK	Buffalo Green Monkey Kidney cells
CI	confidence interval
CPE	cytopathic effect
CSO	combined sewer overflow
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
FWPCA	Federal Water Pollution Control Administration
HAV	Hepatitis A Virus
ICC/nPCR	integrated cell culture/nested polymerase chain reaction
IEC	Interstate Environmental Commission
IPN	Infectious Pancreatic Necrosis
L	liter
mL	milliliter
MPN	most probable number
NF	not found
NOAA	National Oceanic and Atmospheric Administration
NJDEP	New Jersey Department of Environmental Protection
PCBs	polychlorinated biphenyls
PCDD/Fs	polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans
QA/QC	quality assurance and quality control
SDG	sample delivery group
TMDL	total maximum daily load
μm	micron
USGS	U.S. Geological Survey
WWTP	wastewater treatment works

Executive Summary

Exponent has completed a preliminary human health risk assessment for pathogen contamination in the lower Passaic River based on water samples collected from the Saybrook Place combined sewer overflow (CSO) in Newark, New Jersey, on September 23, 2003, shortly after a rainstorm. The samples were collected near the mouth of the CSO pipe and contained levels of fecal bacterial indicators well above both the applicable New Jersey Department of Environmental Protection (NJDEP) water quality standards and U.S. Environmental Protection Agency (EPA) criteria. The surface-water samples also contained many bacteria species that are associated with human fecal matter and are known to cause illnesses, some of which are serious and can be fatal. High levels of the *Giardia* protozoan were also found in the samples at concentrations similar to those found in raw sewage. The analyses for viral pathogens have identified at least one type of viable virus, reovirus, in the CSO discharge samples. Surface-water samples recently collected by the Interstate Environmental Commission (IEC) in the Passaic River also contained elevated levels of fecal bacterial indicators, and these results serve to confirm the data collected by Exponent.

A preliminary human health risk assessment was performed to evaluate three exposure scenarios (recreator, visitor, and homeless person) based on the pathogen data and conservative exposure assumptions. The assessment found a very high risk of gastrointestinal illness resulting from exposures to lower Passaic River surface water affected by the CSO discharge. The levels of fecal coliforms in the surface water affected by the CSO discharge suggest that any recreators, visitors, or homeless individuals exposed to the water discharging from the CSO will contract a gastrointestinal illness. The level of fecal coliform poses gastrointestinal illness risks that are 2 to 150 times higher than the EPA's and NJDEP's acceptable risk (illness rate). The levels of fecal *streptococcus/enterococcus* pose a significant health risk, because there is a 5.5% probability of exposed individuals contracting gastrointestinal illnesses. In other words, if 100 persons were exposed to fecal coliforms at the measured levels, five to six of them would contract gastrointestinal illness. The recreator exposure to *Giardia* from the CSO will result in

at least an 80% risk of contracting a *Giardia* infection. The exposures of visitors and homeless persons to *Giardia* discharged from the CSO will likely result in *Giardia* infection for all these individuals. The risk associated with the identified reovirus is uncertain at present, because additional analyses are needed to determine its concentration and whether the virus is infectious to humans. Initial experiments suggest that there is an infectious virus in the CSO discharge, although it is unknown whether the reovirus, which is present and viable in the discharge, is in fact the infectious virus observed.

The health risks associated with exposure to pathogens pose challenges for environmental and public health professionals beyond those typically associated with environmental pollution. This is due primarily to the acute nature of the illnesses associated with pathogen exposure, compared to the chronic nature of toxicity associated with exposure to typical environmental pollution (e.g., chemicals released into the environment). For example, in 2002, the NJDEP presented estimated risks associated with exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) through the consumption of crabs from the lower Passaic River (NJDEP 2002). Interestingly, the NJDEP completely ignored the health risk posed by other chemicals and pathogens in this assessment. However, the pathogen-related risk estimates presented herein are comparable to, if not greater than, the dioxin risk estimates from NJDEP.

The high level of risk posed by the pathogen discharge into the lower Passaic River is inconsistent with the Clean Water Act policy of protection and propagation of fish, shellfish, and wildlife, and recreation in and on the water, and threatens the current and future use of this water body by the public, regardless of environmental pollution. For example, there is ample documentation in the peer-reviewed scientific literature that fish exposed to pathogens can exhibit a variety of adverse tissue and behavioral effects.

1 Purpose and Background Information

1.1 Purpose

The purpose of this sampling plan and human health risk assessment was to collect and analyze surface-water samples from strategic location(s) in the lower Passaic River Study Area (Study Area) in order to determine whether combined sewer overflow (CSO) outfalls were discharging pathogens into the Study Area and to evaluate the potential human health risk of such discharges.

The remainder of this section provides relevant background information that was used to identify specific pathogens known to be associated with CSO discharges and that pose risks to recreators and other users of a water body. Section 2 provides the details of the sampling plan and concludes with a section that describes how pathogen data can be interpreted from a health risk perspective. Section 3 describes the actual sample collection activities, and Section 4 presents an evaluation of the pathogen data. Section 5 presents a human health risk assessment of the pathogen data that were collected, and Section 6 summarizes the results of this project.

1.2 Background Information

Microorganisms are ubiquitous in terrestrial and aquatic ecosystems. A small subset of these microorganisms is harmful—if taken into the body, they can cause sickness or even death. As a group, these disease-causing microorganisms are known as *pathogens*. Pathogens are a serious concern for managers of water resources. Because of the pathogens' small size, they are easily carried by stormwater runoff or other discharges into natural water bodies. Once in a stream, lake, or estuary, they can infect humans through contaminated fish and shellfish, skin contact, or ingestion of water. Of the designated uses listed in section 303(c) of the Clean Water Act, protection from pathogenic contamination is most important for waters designated for recreation

(primary and secondary contact); public water supplies; aquifer protection; and protection and propagation of fish, shellfish, and wildlife. The presence of pathogens in water bodies is the second leading cause of impairment in the United States (U.S. EPA 2004). As noted in N. J. A. C. 7:9B, the Passaic River is designated as an SE3 water body with the following uses:

1. Secondary-contact recreation
2. Maintenance and migration of fish populations
3. Migration of diadromous fish
4. Maintenance of wildlife
5. Any other reasonable uses.

NJDEP indicates that secondary-contact recreation "means recreational activities where the probability of water ingestion is minimal and includes, but is not limited to, boating and fishing." The NJDEP has determined that the lower Passaic River is an impaired water body due to fish containing elevated concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans (PCDD/Fs) (NJDEP 2004). However, the NJDEP is silent on pathogens for the Passaic River, because it does not list such as an impairment on its 303(c) list (list of impaired water bodies in the state).

1.2.1 Recreational and Seafood Harvesting Use Issues

Excessive amounts of fecal bacteria in surface water used for recreation have been known to indicate an increased risk of pathogen-induced illness to humans. Infections due to pathogen-contaminated recreational waters include gastrointestinal, respiratory, eye, ear, nose, throat, and skin diseases (U.S. EPA 1986). Gastrointestinal symptoms include vomiting, diarrhea, fever, and stomach ache or nausea accompanied by fever. Furthermore, for pathogens such as Giardia,

humans with compromised immune systems may have more a serious and prolonged infection (USEPA, 1999).

In 1982, an outbreak of gastrointestinal illness among New York City police and firefighter scuba divers who swam in the Hudson and East Rivers was investigated (U.S. EPA 1998). It was found that pathogens such as *Entamoeba histolytica* or *Giardia lamblia* were in those water bodies and in 60% of the affected divers.

During July–August 1997, the largest reported outbreak in North America of *Vibrio parahaemolyticus* infections occurred (CDC 1998). Illness in 209 persons was associated with eating raw oysters harvested from California, Oregon, and Washington in the United States, and from British Columbia in Canada; one person died. After closure of the implicated oyster harvest beds, no additional cases associated with eating raw oysters were reported.

During 1997–1998, a total of 18 states reported 32 outbreaks of illness associated with use of recreational waters (CDC 2000). These 32 outbreaks caused illness in an estimated 2,128 persons. Most of the recreational water–related outbreaks reported were associated with swimming pools; however, there were significant outbreaks related to swimming in lakes and rivers. For example, four cases of primary amebic meningoencephalitis attributed to *Naegleria* were reported. All four of the infected children, who ranged in age from 3 to 14 years, died. Infection was acquired when the children swam in a lake (two children), river (one child), or canal (one child). Another example is a reported outbreak of leptospirosis that occurred among competitors in a triathlon in Illinois during 1998. Three hundred seventy-five persons became ill after swimming in a lake. Twenty-eight of them were hospitalized, making this the largest outbreak of leptospirosis ever reported in the United States.

Analyses of disease outbreak reports associated with recreational or occupational contact with marine water have found a dose-response relationship between gastrointestinal symptoms and water quality measured by bacterial counts (Pruss 1998). Human enteric viruses in urban wastewater often flow into the marine environment through sewer outfalls, which can cause

pollution of adjacent beaches used for bathing, and of areas where fish and seafood grow and are harvested for human consumption (Shuval 1988).

1.2.2 Water Quality Criteria for Pathogens

In 1968, criteria were established by the Federal Water Pollution Control Administration (FWPCA) of the U.S. Department of the Interior for fecal coliforms at a level of 200 fecal coliform organisms (colony-forming units when cultured) per 100 mL of water (U.S. EPA 1968). In addition to the presence of fecal coliform bacteria in the water column, many studies have shown the presence and survival of fecal coliforms, as well as pathogens, in marine and freshwater sediments (Nix et al. 1994). A study done in Oak Creek, Arizona, found that water quality violations occurred only when high levels of fecal coliforms were found in the sediments (Crabill et al. 1999). These fecal coliforms may signify the presence of pathogens, which pose a potential health risk. Activities such as recreational swimming that resuspend contaminated sediments and the associated fecal bacteria and pathogens can increase the health risks to persons using these waters.

In 1986, the U.S. Environmental Protection Agency (EPA) published *Ambient Water Quality Criteria for Bacteria—1986.* The data supporting the water quality criteria were obtained from a series of research studies conducted by EPA examining the relationship between swimming-associated illness and the microbiological quality of the waters used by recreational bathers (U.S. EPA 1986). The results of those studies demonstrated that fecal coliforms, the indicator originally recommended in 1968 by the FWPCA, showed less correlation to swimming-associated gastroenteritis than some other indicator organisms. Two indicator organisms, *Escherichia coli* and *enterococci*, showed a strong correlation, the former in fresh waters only and the latter in both fresh and marine waters.

Consequently, EPA's *Ambient Water Quality Criteria for Bacteria—1986* recommends the use of *E. coli* and enterococci, rather than fecal coliforms, to assess water quality. The recommended steady-state geometric mean values of these water quality criteria for bacteria are

33 enterococci per 100 mL and 126 *E. coli* per 100 mL for fresh waters, and a geometric mean of 35 enterococci per 100 mL for marine waters. These values are based on specific levels of risk of acute gastrointestinal illness. The levels of risk used by EPA that correlate to these values are no more than eight illnesses per 1,000 swimmers for fresh waters, and no more than 19 illnesses per 1,000 swimmers for marine waters. The illness rates are EPA's best estimates of the acceptable illness rates for areas that had previously applied the fecal coliform criterion. EPA determined that when implemented in a conservative manner, these water quality criteria are protective of gastrointestinal illness resulting from primary-contact recreation.

New Jersey has water quality standards for fecal coliforms and enterococci specific to types of water bodies (NJDEP 1998), as follow.

Fecal coliform:

- 50 CFU per 100 mL within 1,500 ft of coastal shoreline
- 200 CFU per 100 mL, with less than 10% greater than 400 per 100 mL in freshwater and greater than 1,500 ft of the coastal shoreline and surface water of estuaries designated SE1
- 770 CFU per 100 mL for surface water of estuaries designated as SE2
- 1,500 CFU per 100 mL for surface water of estuaries designated as SE3.

Enterococci:

- 33 CFU per 100 mL, and no single sample greater than 61 per 100 mL for fresh water
- 35 CFU per 100 mL and no single sample greater than 104 per 100 mL for coastal shoreline or estuaries.

The Lower Passaic River from the confluence with the Second River to the mouth at Newark Bay is classified as an SE3 water body and thus falls under the fecal coliform standard of 1,500 per 100 mL.

1.2.3 Sources of Pathogens

1.2.3.1 CSOs

Major point sources of pathogens are discharges from wastewater treatment plants (WWTPs) and CSOs. Raw sewage entering the WWTP typically has a total coliform count of 10^7 to 10^9 most probable number (MPN) per 100 mL (Novotny et al. 1989). Associated with raw sewage are proportionally high concentrations of pathogenic bacteria, viruses, and protozoans.

In some instances, raw sewage can bypass WWTPs and enter water bodies directly. This can occur because of failures or leaks in sanitary sewer systems or, in the case of CSOs, when wet-weather flows exceed the conveyance and storage capacity of the combined system. In CSOs, urban runoff and sanitary sewage are conveyed in the same system. Typical CSO concentrations for total coliforms are reported as 10^5 to 10^7 MPN/100 mL (Novotny et al. 1989), or about 1 order of magnitude greater than treatment plant effluent. In contrast to WWTP effluent, CSOs discharge for short periods of time and at random intervals, and are associated with storm flows that provide dilution of the effluent. Other point sources that can contribute substantial loads of pathogens and fecal indicators to water bodies include concentrated animal feeding operations; slaughterhouses and meat processing facilities; tanning, textile, and pulp and paper factories; and fish and shellfish processing facilities. On the Passaic River, the Passaic Valley Sewerage Commissioners operate a number of CSOs. These CSOs are permitted for operation by NJDEP under a general CSO permit.

1.2.3.2 Nonpoint Sources

A potential nonpoint source of pathogens is the resuspension of bacteria indicators and pathogens in sediments. For example, Weiskel et al. (1996) reported significantly increased values of water-column fecal coliform density after artificial disturbance of the surface 2 cm of sediments in Buttermilk Bay, Massachusetts. The most pronounced increases occurred at sites underlain by fine-grained, high-organic-carbon muds. As runoff during a storm event begins, the discharge and velocity increase, in turn scouring bacteria from the benthic areas of the stream (Yagow and Shanholtz 1998). This scouring causes increased levels of bacteria concentrations in the water column and decreased levels in the stream sediments. After peak discharge, the bacteria concentrations in the water column decrease at a faster rate than in the discharge. This causes the sediment to be deposited downstream, where the sediment bacteria concentrations increase and water-column concentrations return to background levels. The increasing usage of recreational waters can cause resuspension of the high numbers of bacterial indicators and pathogens that occur in the sediments (Burton et al. 1987). This creates a potential health hazard from the possible ingestion of the resuspended pathogens.

1.2.3.3 Water-Body Conditions

After discharge into a water body, pathogenic organisms are subject to many additional factors during dispersion and transport. The factors that influence the survival of the pathogenic organisms within the water body are the physical conditions of the water (Baudisova 1997), sunlight, temperature, salinity, predation, nutrient deficiencies, toxic substances, settling, resuspension of particles with sorbed organisms, and after growth (growth of the organisms in the water body) (Thomann and Mueller 1987). Typically, conditions favorable to the survival of pathogens in water are lower amounts of light energy, lower salinity, elevated levels of nutrients and organic matter, and lower temperatures. These conditions are expected to be consistent with the CSO discharges of interest.

1.2.3.4 Settling

Once settled, pathogens and bacterial indicators can have an increased survival time due to protection from harmful factors such as sunlight and temperature. Fecal coliforms and specific pathogenic organisms have been shown to survive for longer periods of time in sediments than in the overlying water column (Sherer et al. 1992; Burton et al. 1987; Thomann and Mueller 1987). The sediment reservoir allows for the enteric and pathogenic bacteria to survive for up to several months, making resuspension and ingestion in primary-contact waters a significant threat to swimmers (Burton et al. 1987). Increased survival rates for viruses in estuarine sediments have been reported by LaBelle and Gerba (1980), Roper and Marshall (1979), Burton et al. (1987), and Sherer et al. (1992). Due to the accumulation of pathogens in bottom sediments, resuspension of the sediment and the subsequent desorption of the pathogens is a potential source of contamination to the overlying water. A study by Sherer et al. (1992) showed the survival of fecal coliform and fecal streptococci to be significantly longer in sediment-laden waters than in waters without sediment. Fecal coliform and fecal streptococci bacteria showed half-lives from 11 to 30 days and 9 to 17 days, respectively, when incubated with sediment. These are longer half-lives than those when sediment was not present. During the study, the stream bottom was disturbed several times. The mean concentration of fecal coliform in the stream increased by 1.7 times the initial concentration after the stream bottom was disturbed. The fecal streptococci concentration increased by 2.7 times. This study showed that enteric bacteria can survive in sediments for several months, as compared to only a few days in the overlying water. Bothner et al. (1994) reported finding fecal coliforms in sediments collected from a deep ocean dump site off New York.

1.2.4 Pathogen Types

Pathogens most commonly identified and associated with waterborne diseases can be grouped into three general categories: bacteria, protozoans, and viruses.

1.2.4.1 Bacteria

Bacteria are unicellular organisms that lack an organized nucleus and contain no chlorophyll . They contain a single strand of DNA and typically reproduce by binary fission, during which a single cell divides to form two new cells. Wastes from warm-blooded animals are a source for many types of bacteria found in water bodies, including the coliform group and *Streptococcus, Lactobacillus, Staphylococcus*, and *Clostridia*. Not all bacteria are pathogenic, however. Table 1 presents information on some of the major pathogenic waterborne bacteria of concern.

Table 1. Major pathogenic waterborne bacteria[a]

Bacteria	Disease	Effects
Escherichia coli	Gastroenteritis	Vomiting, diarrhea
Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of the small intestine
Salmonella	Salmonellosis	Diarrhea, dehydration
Shigella	Shigellosis	Bacillary dysentery
Vibrio cholerae	Cholera	Extremely heavy diarrhea, dehydration
Yersinia enterolitica	Yersinosis	Diarrhea

[a] Adapted from U.S. EPA (2001)

1.2.4.2 Protozoans

Protozoans are unicellular organisms that reproduce by fission and occur primarily in the aquatic environment. Pathogenic protozoans constitute almost 30% (or 10,000) of the 35,000 known species of protozoans (Mitchell et al. 1988, cited in NCSU 1997). Pathogenic protozoans exist in the environment as cysts that hatch, grow, and multiply after ingestion, manifesting as the associated illness. Encystation of protozoans facilitates their survival, protecting them from harsh conditions such as high temperature and salinity. Two protozoans of major concern as waterborne pathogens are *Giardia lamblia* and *Cryptosporidium*. *Giardia* causes giardiasis, one of the most prevalent waterborne diseases in the United States; *Cryptosporidium* causes cryptosporidiosis. Because infection with *Cryptosporidium* can occur after swallowing as few as 10–100 oocysts, swallowing a single mouthful of contaminated water from recreational swimming could cause illness (CDC 2000). Some waterborne protozoans

from fecal sources that pose threats to human health are listed with their associated diseases in Table 2.

Table 2. Major pathogenic waterborne protozoans[a]

Protozoan	Disease	Effects
Balantidium coli	Balantidiasis	Diarrhea, dysentery
Cryptosporidium	Cryptosporidiosis	Diarrhea, death in susceptible populations
Entamoeba histolytica	Amebiasis (amoebic dysentery)	Prolonged diarrhea with bleeding; abscesses of the liver and small intestine
Giardia lamblia	Giardiasis	Mild to severe diarrhea, nausea, indigestion

[a] Adapted from U.S. EPA (2001)

1.2.4.3 Viruses

Viruses are a group of infectious agents that require a host in which to live. They are composed of highly organized sequences of nucleic acids, either DNA or RNA, depending on the virus. All viruses have a protein covering that encloses the nucleic acid. Some viruses have a lipoprotein (protein in which at least one of the components is a lipid) envelope over the protein covering. The protein or lipoprotein covering determines to what surface the virus will adhere. The most significant virus group affecting water quality and human health originates in the gastrointestinal tract of infected individuals. These *enteric viruses* are excreted in feces and include hepatitis A, rotaviruses, Norwalk-type viruses, adenoviruses, enteroviruses, and reoviruses. Table 3 presents some important viruses and their associated diseases.

Table 3. Major pathogenic waterborne viruses[a]

Virus	Disease	Effects
Adenovirus	Respiratory disease, gastroenteritis	Various effects
Enterovirus	Gastroenteritis, heart anomalies, meningitis	Various effects
Hepatitis A	Infectious hepatitis	Jaundice, fever
Reovirus	Gastroenteritis	Vomiting, diarrhea
Rotavirus	Gastroenteritis	Vomiting, diarrhea
Calicivirus	Gastroenteritis	Vomiting, diarrhea
Astrovirus	Gastroenteritis	Vomiting, diarrhea

[a] Adapted from U.S. EPA (2001)

1.2.5 Indicator Organisms

Scientists and public health officials typically choose to monitor nonpathogenic bacteria that are usually associated with pathogens transmitted by fecal contamination but are more easily sampled and measured. These associated bacteria are called *indicator organisms*. Indicator organisms are assumed to indicate the presence of human pathogenic organisms. When large fecal coliform populations are present in the water, it is assumed that there is a greater likelihood that pathogens are present (McMurray et al. 1998). Fecal indicators are used to develop water quality criteria to support designated uses, such as primary-contact recreation and drinking-water supply.

There has been a resurgence of interest in the enterococcus group as indicators (Davies-Colley et al. 1994). Enterococci (a subgroup of the fecal streptococci group) are round, coccoid bacteria that live in the intestinal tract. *Streptococcus faecalis* and *Streptococcus faecium* (part of the enterococci family) are thought to be more human-specific than other streptococci, but they can be found in the intestinal tracts of other warm-blooded animals such as cats, dogs, cows, horses, and sheep. The risk to swimmers of contracting gastrointestinal illness seems to be predicted better by enterococci than by fecal coliform bacteria, because the die-off rate of fecal coliform bacteria is much greater than the enterococci die-off rate.

The 1986 federal bacteriological water quality criteria document (U.S. EPA 1986) critically reviewed a series of epidemiological and water quality monitoring studies at marine and freshwater beaches since 1972. A comparison of various fecal indicators of potential pathogens with disease incidence revealed that elevated levels of enterococci bacteria were most strongly correlated with gastroenteritis in both fresh and marine recreational waters. The gastroenteritis was assumed to be related to the elevated levels of enterococci. *E. coli* also showed a correlation with gastroenteritis, primarily in fresh water.

A review by Pruss (1998) of 22 studies of recreational waters showed that the indicator organisms that correlate best with illness are enterococci/fecal streptococci for both marine

water and fresh water, and *E. coli* for fresh water. The microbiological indicators yield a general assessment of water quality and safety for the designated or existing use and do not identify specific human pathogens; that is, the exceedance of criteria developed for *E. coli* and enterococci bacteria indicates that the water *might* cause some type of illness following exposure to that water. For example, recreational use by swimmers or surfers could be impaired by the presence of high densities of fecal indicators, because there is a chance that some of those microorganisms could cause gastrointestinal illnesses if the water is swallowed. Commercial or recreational harvesting of clams in an estuary could be impaired, because the presence of high densities of these bacteria suggests that other human pathogens, such as the infectious hepatitis A virus, might be present in the shellfish tissues.

1.2.6 Pathogen Effects on Fish

Pathogens have been reported to cause adverse effects in fish. While pathogens cause harmful effects both internally and externally in fish, the most observable effects relate to skin diseases. Presented below is a brief summary of the reported effects of pathogens on fish.

1.2.6.1 Effects of Bacteria on Fish

Fish tuberculosis is a systemic disease characterized by the production of focal granulomas due to presence of bacteria from the genus *Mycobacterium*. *M. marinum*, *M. chelonei,* and *M. fortuitum* are the most common *Mycobacterium* species involved. This disease has been reported in 40 families and 151 species of fish worldwide and is known to be present in freshwater and saltwater fish in both tropical and temperate zones (Ribelin and Migaki 1975). The aquatic environment is believed to be the source of initial infection, with fish becoming infected by ingestion of bacteria-contaminated food or debris (Moeller 2001). Microscopic examination reveals focal granulomas composed of epithelial cells and hystiocytes occupying a central position surrounded by a wall of fibroblastic cells. Typically, affected fish are emaciated, ascetic, may have ulcerations, exophthalmus (bulging of eyes), lordosis or scoliosis,

and, frequently, pigment changes. However, an acute form of fish tuberculosis is known that may result in death with few or none of these symptoms. Gross lesions include grayish-white raised areas of various sizes involving any organ. Focal granulomas can form conglomerate lesions, completely destroying the organ they occupy (Ribelin and Migaki 1975). Often, numerous acid-fast bacteria are observed in the granulomas (Moeller 2001).

Many bacteria localize in the kidney, partly due to the extensive blood supply and trapping abilities of the organ. *Pseudomonas sp.* are known to damage this area of diseased fish (Ferguson 1992). Bacteria belonging to the genus *Pseudomonas* are present in most natural waters and infect most species of fish. These parasites are considered opportunistic pathogens, causing disease when the host is subjected to some type of stress. Clinical symptoms include hemorrhages in the mouth region, opercula, and ventral side of the body. Small petechial hemorrhages can occur throughout the body cavity. The liver may also be affected. *Pseudomonas spp.* have been credited with causing *Pseudomonad septicemia*, red spot disease, fin/tail rot, and others (MA CZM 1995).

Hemorrhagic septicemia is a systemic disease associated with the bacterium *Aeromonas hydrophilia* and other species of the genus *Pseudomonas*. *A. hydrophilia* is a common water saprophyte with a great variation in virulence in serotypes (Moeller 2001). Disease associated with *Pseudomonas sp.* is transmitted via contaminated water or diseased fish. Diagnosis is rendered by culturing the organism from affected animals (Moeller 2001). The disease is characterized by dermal ulceration, ascites, and necrosis of major organs. Layers of the epidermis in diseased fish are known to have extensive intra- and extra-cellular edema, and capillaries can be extremely congested. This disease is found worldwide and affects a variety of fish, including teleosts and elasmobranches. It is a disease of freshwater fish, but the genus has been detected in water of 7 ppt salinity or less. Synonyms for this disease include rubella (all fish), hydropigenous viral neurosis (all fish), infectious abdominal dropsy (carp), myoenterohepatic syndrome (carp), redmouth (trout), red pest (pike), and red sore (pike) (Ribelin and Migaki 1975).

Microsporidians, such as *Glugea sp.*, *Loma sp.*, and *Pleistophora sp.*, are bacteria that cause Microsporidiosis. They are found in numerous fresh and saltwater fish and form cysts in various organs. *Glugea stephani* was first reported in the NY/NJ Harbor Estuary in winter flounder in 1981, and are now present in numerous fish species throughout the year (NJ MSC 1987).

Vibriosis is an acute, systemic disease of fish characterized by dermal ulceration, hematopoietic necrosis, and anemia due to the presence of the bacterium *Vibrio anguillarum. Vibrio* species are present in a large number of fish species worldwide, including fish in the NY/NJ Harbor Estuary (NJ MSC 1987). It is primarily a saltwater fish disease; however, there are known accounts of this disease in freshwater environments. Outbreaks of this disease can be epizootic and cause impacts to recreational or commercial fishing. Bacterial disease caused by *Vibrio spp.* may result in uveal lesions (Ferguson 1992). Characteristic gross lesions associated with outbreaks of vibriosis affect the skin and include reddening at the base of the fins, petechiae, ecchymoses, fluctuating vesicles, and ulceration. Other gross lesions include fin rot, ascites, congestion of spleen and liver, swelling of the kidney, intestinal hyperemia, and a clear viscous fluid within the gut. Necrosis of the dorsal and ventral fins is present in the majority of cases (Ribelin and Migaki 1975).

Bacteria of the three genera *Vibrio*, *Aeromonas*, and *Pseudomonas* are commonly associated with fin necrosis (i.e., fin rot) in a large number of fish species worldwide, including fish from the NY/NJ Harbor Estuary (NJ MSC 1987). Other external pathological effects of these bacteria include skin hemorrhages and skin ulcers (Moeller 2001).

Various forms of bacteria and viruses can cause oral congestion and hemorrhages of the mouth in fish species. The enteric redmouth bacterium, *Yersinia ruckeri*, is one such bacterium known to cause ecchymotic hemorrhaging in diseased salmon species (Ferguson 1992).

1.2.6.2 Effects of Viruses on Fish

Lymphocystis is one of the most documented viral diseases that cause skin lesions in both marine and freshwater fish (Sindermann 1970). First described in European flounder, this disease has been reported in more than 49 species, including mummichog and striped bass (Sindermann 1970). This disease may be present in fish populations at all times, but may periodically increase in intensity due to overcrowding and/or environmental degradation (NJ MSC 1987). Infectious Pancreatic Necrosis (IPN) is a viral disease caused by the Rotifer birnavirus, *Birnaviridae.*

Viral infection by birnavirus is characterized by a sudden explosive outbreak with high mortality. Affected fish become dark and rotate their bodies while swimming. Diseased fish usually have distended abdomens and exophthalmus. The presence of a gelatinous material in the stomach and anterior intestine is highly suggestive of IPN (Moeller 2001).

Adenoviruses and rotaviruses such as astroviruses, caliciviruses, and reoviruses have been documented in fish and shellfish for human consumption, but effects on aquatic organisms are unknown.

1.2.6.3 Effects of Protozoa on Fish

Ichthophthirius multifiliis is one of the most common protozoan infections in freshwater fish. The ciliate protozoans are large enough to be visible to the unaided eye, and their multifocal distribution brings about their commonly known name, "white spot disease" (Ferguson 1992). *Cryptocaryon irritans* is the saltwater equivalent to *I. multifiliis*. Pathogenic effects include increased mucus production in gills and skin, clouded eyes, and white spots on the skin. Fish mortality can occur from oxygen deprivation due to hyperplasia of the gill epithelium (Moeller 2001). The ciliate protozoan *Balantidium* is associated with disease in Cyprinid fish species (Ferguson 1992). *Cryptosporidium* and *Giardia* are two common protozoa that occur in human and animal wastes, and reach aquatic habitats through surface runoff and sewage and

wastewater discharges to receiving water. *Cryptosporidia* have been reported in fish, but their effects are not documented in reviewed literature (Ferguson 1992).

2 Sampling

The sampling plan was based on the general background information described above and site-specific factors related to the Passaic River Study Area. The methods and procedures for sample collection, processing, and analysis followed the site-specific standard operating procedure, as well as EPA and U.S. Geological Survey (USGS) guidance as outlined in the following documents:

- Saybrook Place Combined Sewer Outfall Surface Water Pathogen Sampling Standard Operating Procedures (Appendix A)
- National Field Manual for the Collection of Water-Quality Data (USGS 2002)
- Protocol for Developing Pathogen TMDLs (U.S. EPA 2001)
- Microbiological Monitoring for the U.S. Geological Survey National Water-Quality Assessment Program (USGS 2000).

In addition, information obtained from USGS staff who are actively involved in pathogen sampling was incorporated into the sampling plan.

The hours of potential sampling were limited to Monday through Thursday during normal business hours (9 a.m. to 5 p.m.), to ensure that the samples were delivered to the laboratories within the required holding times (Table 4).

2.1 Sample Locations

The Saybrook Place CSO was targeted for this initial round of sampling. This site was selected because of its location (within the Passaic River Study Area) and the relative ease of access to the CSO for the sampling team.

2.2 Methods

2.2.1 Sample Identification

Sample identifiers were assigned to each sample as it was collected. Each container was assigned a sample number. Each assigned sample number was a predetermined, sequential number linking the sample descriptive information recorded in the field log book and chain-of-custody documentation. A preprinted label containing the sample number and the type of laboratory analysis was affixed to the sample container and covered with clear plastic tape. The format of the sample identifier was:

- CSOnn-SAY-date (e.g., CSO01-SAY-061203).

The samples collected for this CSO were grouped under the following sample data group (SDG) identifier:

- PATH-CSO-01.

2.2.2 Sample Collection

Two sets of water samples were collected at each location: one set from the CSO itself (sampled from river access), and one set from the surface water within 10 ft of the discharge point. Samples were collected using the hand-dip method (Appendix A, USGS 2000) as soon as possible after the onset of rainfall. Separate samples were needed for the different analyses; therefore, each sample set consisted of a minimum of 50.3 L of water collected in pre-cleaned or sterile bottles provided by the laboratories (see Section 2.2.5 and Table 4). Decontamination procedures were followed as described in the site-specific SOP (Appendix A). Field collection activities are described in Section 3 below.

2.2.3 Sample Packaging and Handling

The analytical laboratories were alerted when the samples were prepared for shipment. Sample packaging and handling were conducted in accordance with standard operating procedures (SOPs) outlined in *Passaic River Surface Water Pathogen Sampling Sample Packaging and Handling Standard Operating Procedure* (Appendix A). In addition, the following measures were taken in accordance with USGS guidance (USGS 2000, 2002) to ensure the proper handling of all samples. Upon collection, samples were chilled at 1 to 4 °C and stored in the dark until analysis. Extra care was taken to ensure that samples were not allowed to freeze and that sample bottles were not completely submerged in water from melting ice. The holding time for samples to be analyzed for bacteria did not exceed 6 hours (USGS 2000).

2.2.4 Field Documentation

All samples and field sampling procedures were recorded in accordance with standard operating procedures (SOPs) outlined in SOP GEN-01, *Field Documentation* (Appendix A) and SOP GEN-02, *Sample Custody* (Appendix A). The field team leader was responsible for properly completing all logbooks and forms. Station and sample logs were completed at the time the observations were made. Field notes are included in Appendix B.

2.2.5 Laboratory Analysis

In accordance with USGS guidance, analysis of samples for bacteria began within 6 hours of collection, and analyses for the remaining organisms began within 72 hours. Samples were analyzed for the organisms discussed below.

Bacteria:

- Total coliforms include several genera of bacteria that occur naturally in human and animal intestinal tracts, as well as in soils, fresh water, and marine waters. Because of the variety of sources of total coliforms, the significance of their presence is questionable. Therefore, they are generally used as a rough estimate of water quality and as a screen for fecal coliforms (USGS 2000).

- Fecal coliforms is a subset of total coliforms. These bacteria are found in the intestines and feces of warm-blooded animals. However, nonfecal sources have been determined for at least one species of this group (USGS 2000).

- *E. coli* is a species of fecal coliform bacteria and is considered a direct indicator of fecal contamination from warm-blooded animals (U.S. EPA 2001; USGS 2000).

- Fecal streptococci are found in the gastrointestinal tract of warm-blooded animals. However, at least one species has been found to exist for extended periods in soil and water. Enterococci is a subgroup of the fecal streptococci that is considered a more specific indicator of fecal contamination (USGS 2000). For this task, fecal streptococci were measured and assumed to be representative of enterococci.

- *Clostridium perfringens* is considered a better indicator bacterium for enteric pathogenic endospore-forming bacteria and protozoans, because its spores are

more resistant to disinfection and environmental stresses than those of *E. coli* (USGS 2000).

- *Pseudomonas aeruginosa* has the ability to adapt to and thrive in many environments, such as water and soil, and plant and animal tissue. It has a remarkable capacity to cause disease in susceptible hosts, but rarely infects healthy individuals. *P. aeruginosa* is a major cause of hospital-acquired infections such as pneumonia, pharyngitis, sepsis (bloodstream infection), and urinary-tract infections, and is highly resistant to antibiotics.

- *Klebsiella* can be found in the gastrointestinal tract. *Klebsiella* infections are usually hospital-acquired and occur in people with a weakened immune system. The most common infection caused by *Klebsiella* is pneumonia; other infections include bronchitis, surgical wound infection, bacteremia (infection of the blood), and urinary tract infection. *Klebsiella* bacteria are generally resistant to most antibiotics.

- *Staphylococcus aureus* is commonly found in the human nose and on the skin. If *S. aureus* gets into the body, it can cause infections such as pneumonia, blood infection, and skin infections.

Protozoans:

- *Giardia* and *Cryptosporidium* are pathogenic protozoans that produce resistant forms, allowing them to survive harsh environmental conditions (U.S. EPA 2001). These protozoans are responsible for most waterborne illnesses.

Viruses:

- Coliphage (a virus that infects and replicates in fecal bacteria) is an indicator virus often used for assessing risks associated with pathogenic enteric

viruses, because testing for enteric viruses can be both time-consuming and difficult (USGS 2000). Because coliphages are viruses and their physio-chemical properties (size, surface charges, etc.) reflect human enteric viruses better than do coliform bacteria, they are more likely than bacteria to respond to factors affecting transport kinetics than are enteric viruses, and therefore may be better indicators of viral distribution in a water body than bacteria.

- Enteric viruses of interest include adenovirus, astrovirus, rotavirus, and Hepatitis A virus. The standard method (ICR method) requires specialized sample collection apparatus, large sample volume, and time-consuming culturing and analysis.

Immediately after collecting the samples for bacteria analysis, sampling personnel transported them by car to EMSL Analytical at Westmont, New Jersey. EMSL is located approximately 82 miles from Newark, New Jersey. The samples for protozoans analyses were shipped via overnight courier (FedEx) to Analytical Services Inc. (ASI) at Williston, Vermont. The samples for virus analysis were sent to the ASI-affiliated laboratory at the University of New Hampshire in Durham, after being chilled in a refrigerator overnight. A complete list of the target organisms and analytical methods used is presented in Table 4.

Table 4. Target organisms, analytical laboratories, and methods

Target Pathogen	Laboratory	Method	Qualitative or Quantitative	Sample Volume	Sample Hold Time
Aerobic bacteria[1*]	EMSL Analytical[a]	Standard Plate Count with ID	Quant./Qual.	100 mL grab[4]	6 hrs
Fecal coliforms*	EMSL Analytical	Standard Methods 9222[b]	Quant.	100 mL grab[4]	6 hrs
Fecal Streptococcus/ Enterococcus Group D*	EMSL Analytical	Standard Methods 9230	Quant.	100 mL grab[4]	6 hrs
Giardia and *Cryptosporidium*^	Analytical Services[c]	U.S. EPA Method 1623	Quant.	10 L grab[4]	72 hrs
Viruses[2^]	Analytical Services	ICC/nPCR[d] with TCV/MPN[3]	Quant.	5 L grab[4]	72 hrs
Coliphage^	Analytical Services	U.S. EPA Method 1602	Quant.	1 L grab[4]	72 hrs

[1] The method provides a total aerobic bacteria count and identification of species present (e.g., *E. coli* and others).

[2] Enteroviruses (Pan-entero primers: coxsackie, echo, and polio), Adenovirus, Astrovirus, and Rotavirus.

[3] Integrated Cell Culture / Nested Polymerase Chain Reaction with Total Cultivatible Virus/Most Probable Number. Qualitative identification of Pan-entero primers (coxsackie, echo, and polio), Adenovirus, Astrovirus, and Rotavirus with quantitation of Pan-entero primers as a group.

[4] Each sample in a separate container.

[a] EMSL Analytical, Inc. 107 Haddon Ave, Westmont, NJ 08108. Tel: 856-858-4800.

[b] American Public Health Association. 1999. Standard Methods for Examination of Water & Wastewater 20th Ed.

[c] Analytical Services, Inc. 130 Allen Brook Lane, Williston, VT 05495. Tel: 800-723-4432. Affiliated laboratory: Dr. Aaron Margolin. University of New Hampshire. 46 College Road, Durham, NH 03824. Tel: 603-862-4095.

[d] Chapron, C.D., M.A. Ballester, J.H. Fontaine, C.N. Frades, and A.B. Margolin. 2000. The detection of Astrovirus, Enteroviruses, and Adenovirus type 40 and 41 in surface waters collected and evaluated by the Information Collection Rule and an Integrated Cell Culture / Nested PCR Procedure. Appl. Environ. Microbiol. 66(6):2520–2525.

*Sealed 100-mL pre-sterilized transparent plastic bottles to be used for sample collection.

^Ten-liter collapsible transparent plastic bottles to be used for sample collection (not sterilized, because sterilization is not needed).

2.2.6 Quality Assurance/Quality Control

Quality assurance and quality control (QA/QC) procedures in the field sampling were followed in accordance with SOPs outlined in *Passaic River Surface Water Pathogen Sampling Sample Packaging and Handling Standard Operating Procedure,* SOP GEN-01, *Field Documentation,* and SOP GEN-02, *Sample Custody* (Appendix A). For the purposes of this preliminary sampling event, QA/QC samples such as field and method blanks for data validation were not required.

2.3 Data Interpretation

The data obtained from this sampling event can be used to estimate increased disease incidence in a potentially exposed recreational population. There are several regulatory guidance documents and publications in the peer-reviewed literature that describe quantitative methods for estimating disease incidence as a result of oral and dermal contact with pathogens. These include the EPA's Total Maximum Daily Load (TMDL) document and a recently released EPA document titled, "National Beach Guidance and Required Performance Criteria" (U.S. EPA 2001). The U.S. Food and Drug Administration (FDA) also has developed guidance for assessing disease incidence as a result of ingestion of pathogens (the 1991 "Foodborne Pathogen and Natural Toxins Handbook"). The pathogen risk assessment paradigm is similar to the chemical risk assessment process, in that exposure estimates are coupled with dose-response information to determine an outcome. In chemical risk assessments, toxicity criteria (reference doses and slope factors) are developed from the dose-response information. Similarly, in pathogen risk assessment, the exposure estimate (e.g., milliliters of water swallowed per day) is coupled with the concentration data (pathogens pre milliliter) to develop an estimate of pathogen dose. The dose is then compared to dose-response information for that pathogen to determine how many infections might occur in a given population.

Plausible estimates were made of recreational contact with CSO discharge and surface water in the Study Area; these estimates were consistent with EPA's standard exposure assumptions. The data were coupled with the exposure estimates to derive an estimate of disease incidence, as described above. Only qualitative results were available for some of the pathogens (i.e., whether it was present or not), and for these organisms, it was not possible to derive an estimate of disease incidence.

3 Field Activities

Heavy rainfall of sufficient intensity to cause an overflow reached the Newark, New Jersey, area on the morning of September 23, 2003. The project team determined that the rainfall condition could result in discharge from the Saybrook Place CSO, and the sampling team was mobilized. Figure 1 shows a view, from the street level, of the CSO pipe and the floating platform that supports the debris-catching screens.

Figure 1. Saybrook Place combined sewer overflow (CSO)

The National Weather Service station at the Newark Liberty International Airport reported that 0.91 inch of precipitation fell during that day, and the maximum one-hour precipitation of 0.57 inch occurred between 8 and 9 a.m. (NCDC 2003a,b). The 30-year average rainfall for this

date is 0.13 inches (NCDC 2003c). The reported hourly precipitation is shown in Figure 2. The samples were collected during the noon hour. A review of the National Oceanic and Atmospheric Administration's (NOAA's) tidal prediction chart indicated that this time was a low-tide condition (NOAA 2003).

This section and those that follow summarize the sample collection effort, laboratory findings on the pathogen contents in those samples, and a preliminary pathogen risk assessment.

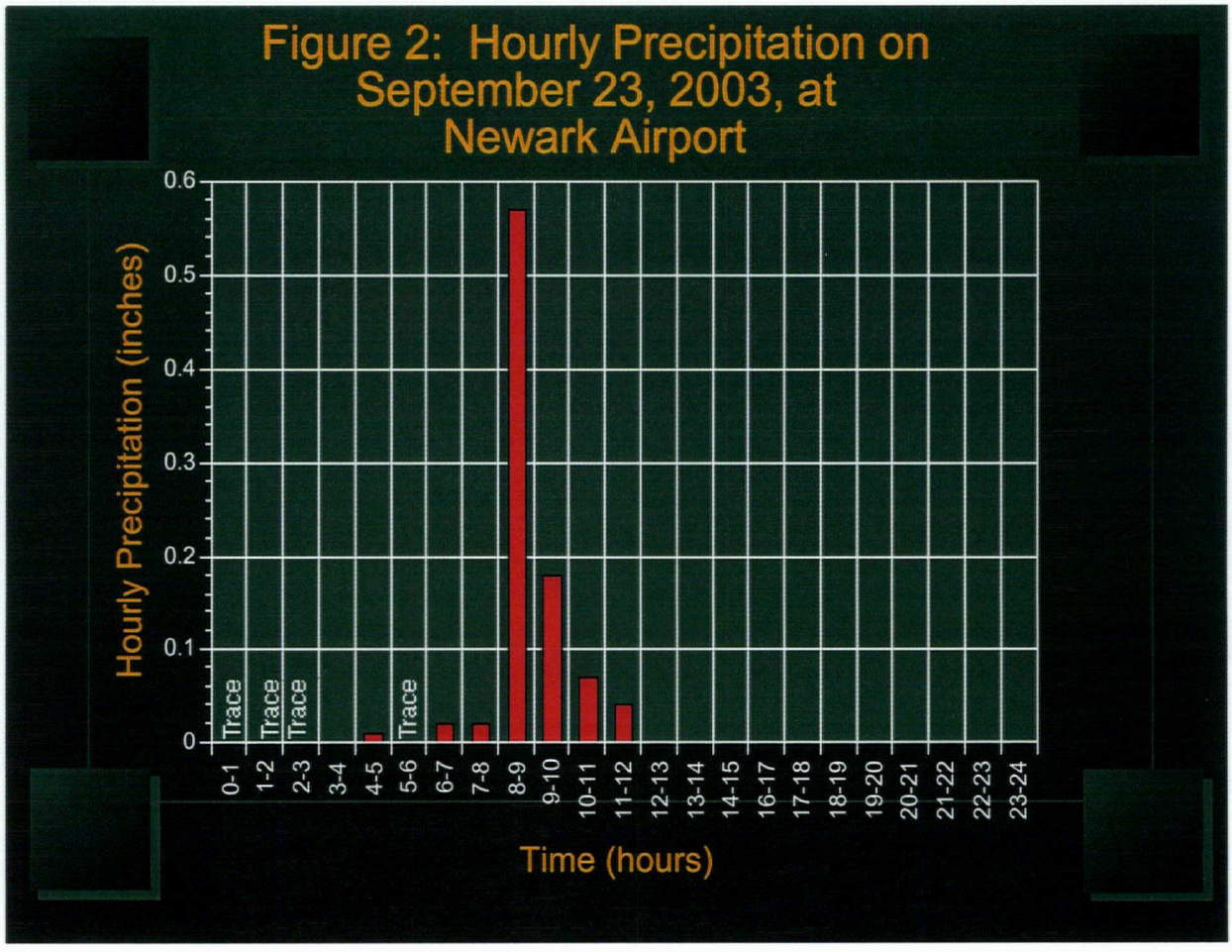

Figure 2. Hourly precipitation on September 23, 2003, at Newark Airport

The sampling team consisted of Steave Su of Exponent and Dan Gardner of Converse Consultants. The sampling team followed the protocols provided in Appendix A.

Two surface-water samples were collected, at 12:15 and 12:30 p.m., respectively. The CSO pipe appeared to be approximately half-submerged, with roughly 3 ft of the pipe above the water surface. During the sample collection, light to moderate rain was falling. Discharge from the CSO pipe was clearly visible and audible (loud gushing), even from the street level (about 30 ft above the pipe). Preliminary measurements of water flow rates using a Horiba sensor and a flow probe showed that the water flow out of the CSO pipe was nearly two-fold higher than the flow rate in the river about 20 ft away from the pipe.

The first sample, CSO01-SAY-092303, was collected at the center of the CSO pipe (Figure 3). The sample was collected by lowering a pre-sterilized 5-gallon bucket tied to a rope from the top of the CSO pipe into the center of the discharge stream. The collected content was poured into a pre-sterilized 32-gallon container until the larger container was roughly three-quarters full. The sample appeared to be semi-transparent, beige liquid.

The second sample, CSO02-SAY-092303, was collected fro the river at a location approximately 10 ft downstream from the CSO pipe (Figure 4). The sample was collected by lowering a pre-sterilized 5-gallon bucket into the river from the platform. The collected content was poured into a pre-sterilized 32-gallon container until the larger container was roughly three-quarters full. The second sample also appeared to be semi-transparent, beige liquid, and it was not visibly different from the first sample. At the end of collecting the second sample, the rain had stopped, but the CSO continued to visibly and audibly discharge.

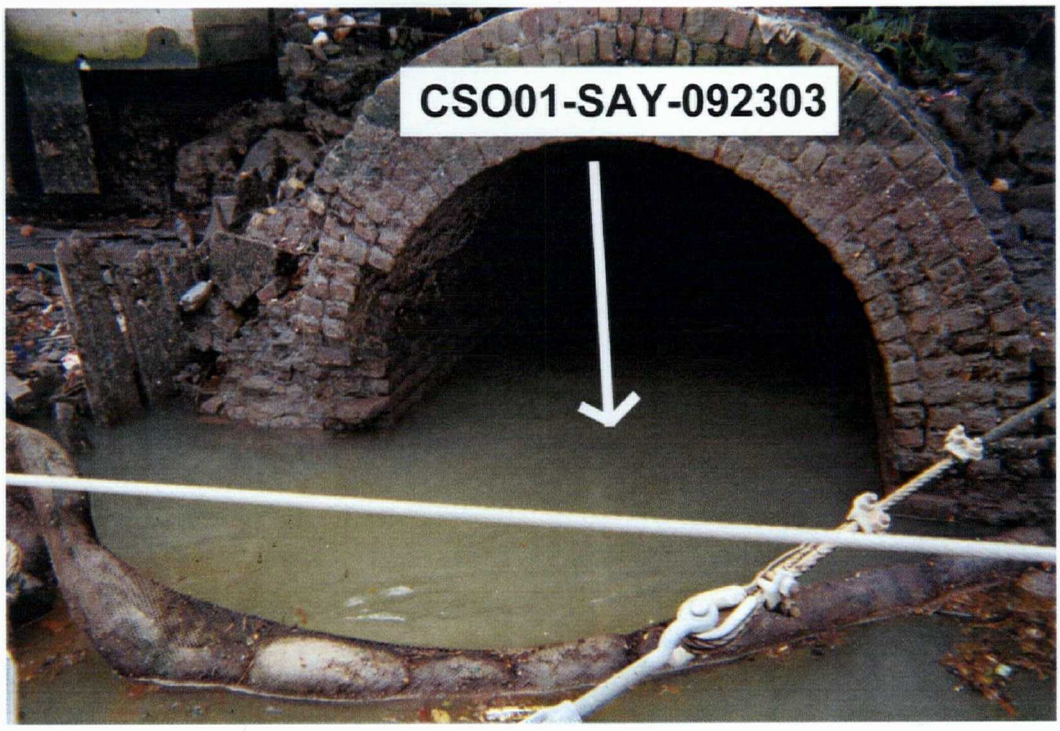

Figure 3. CSO01-SAY-092303 sampling location

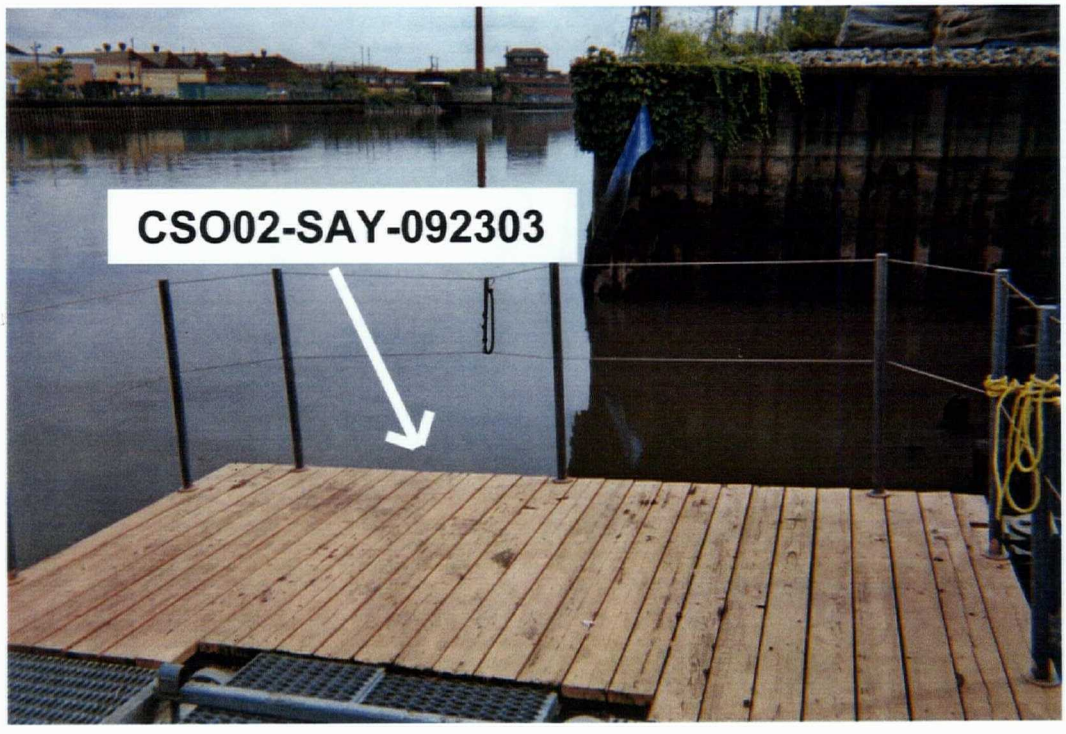

Figure 4. CSO02-SAY-092303 sampling location

The samples were homogenized by shaking the large containers vigorously. From each of the large containers, samples were divided into one 100-mL and ten 10-L containers provided by the laboratories.

The pre-collapsed 10-L containers were difficult to fill in the field. Some of the previously prepared sample tag labels were not usable due to soaking and had to be re-prepared. The transport of samples and equipment back to the street level was also time consuming. Roughly two hours passed between completion of sampling and departure from the site.

Physical parameters were recorded at the two sampling locations using a Horiba sensor and a flow probe and are summarized in Table 5.

Table 5. Physical parameters of water quality at sampling locations

Parameter	Sample	
	CSO01-SAY-092303	CSO02-SAY-092303
Flow rate (m/sec)	0.7	0.4
pH	7.41	7.46
Conductivity (μS/sec)	0.327	0.30
Turbidity (NTU)	80	81
O_2 (mg/L)	0.38	0.15
Temperature (°C)	22.1	21.6
Salinity (%)	0.01	0.01

The two 100-mL samples were hand-delivered to EMSL Analytical Laboratory within the required holding time for bacteria analysis (holding time is 6 hours). The ten 10-L samples were brought to Converse Consultant's facility for overnight chilling and were then taken to FedEx the next day for shipment to the Analytical Service Inc. (ASI) laboratories. On September 24, 2003, separate coolers containing the 10-L samples were shipped to each of the ASI laboratories. The ASI laboratories in Vermont and at the University of New Hampshire

received the ten 10-L samples on September 25, within ASI's required holding time for the protozoa and virus analyses (holding time is 72 hours).

4 Evaluation of Laboratory Results

4.1 Data Quality Review

The data received from EMSL and Analytical Services Inc. were reviewed by Environmental Data Services (EDS). EDS reviewed items such as holding-time requirements, temperature of the samples when received by the laboratory, method compliance, and others. The review by EDS found no significant data quality issues with the pathogen data. The report by EDS can be found in Appendix D.

4.2 Bacteria

EMSL Analytical Laboratory performed analyses to quantify total coliform, fecal coliform, fecal *streptococcus/enterococcus,* and other major bacteria species. The analytical methods were Standard Methods 9222B, 9222D, 9222G, and 9230C—modified membrane filtration methods (EMSL 2003a). Total coliform, fecal coliform, and fecal streptococcus/enterococcus were found to be present in both CSO samples at concentrations greater than 30,000 CFU/100 mL (colony-forming units per 100 mL of water). These results clearly indicate that the CSO pipe discharge and river water at 25 ft downstream of the pipe resembled raw sewage, and the water contained a number of pathogenic bacteria. However, typical CSO concentrations for total coliforms have been reported as 10^5 to 10^7 MPN/100 mL (Novotny et al. 1989), suggesting that the samples collected for this project did not represent the "first flush." The meteorology data described in Section 3 would tend to confirm this.

EMSL reported the presence of numerous other bacteria, including *Citrobacter freundii* at 3,000 CFU/mL. The results and background information on the bacteria detected for the two samples are shown below in Table 6. Laboratory data reports can be found in Appendix C.

4.3 Protozoa

ASI's laboratory in Williston, Vermont, performed analyses to quantify *Giardia* and *Cryptosporidium* using EPA Method 1623 with internal positive control (ASI 2003a). The results for the two samples are presented in Table 7.

Giardia cyst levels that have been reported generally are on the order of 10^3/L to 10^4/L in raw sewage, 10/L to 10^2/L in secondary treated wastewaters, and <10/L in surface waters (U.S. EPA 1998). The levels found for the two samples indicate that the CSO pipe discharge and the surface water roughly 25 ft downstream are equivalent or nearly equivalent to cyst levels in raw sewage. The matrix spike recovery values reported for the *Giardia* analyses were below 15%, suggesting that the Giardia results are biased low (ASI 2003a).

No *Cryptosporidium* was detected in the samples. The lack of *Cryptosporidium* detection may be due to the low analytical recovery, as indicated by the low matrix spike recovery percentages. The established recovery criterion for *Cryptosporidium* is 13%–143%, and the recovery of Sample CSO02-SAY-092303 was below this range (ASI 2003a).

The low recovery percentages suggest that the reported values for *Giardia* are biased low due to matrix interference (i.e., actual cyst levels in the samples were likely to be higher). The ASI lab indicated that low recovery is not unexpected for samples associated with industrial or domestic sources, or raw sewage. Several factors may explain the low recovery. For example, any presence of iron contents in the samples may affect the enrichment/purification step of the analysis, because the procedure involves using immunomagnetic separation of the pathogens (ASI 2003b). The EPA also indicates that available *Giardia* analytical methods provide only 10%–20% recovery (U.S. EPA 1998).

In the United States, levels of *Giardia* reported in water are usually lower than *Cryptosporidium* levels, while in other countries (e.g., Canada), surveys have found higher levels of *Giardia* than *Cryptosporidium* (U.S. EPA 1998). The ASI lab found it unusual for these samples to contain

Giardia and no *Cryptosporidium*, but the low recovery may explain not finding *Cryptosporidium* (ASI 2003b). Laboratory data reports can be found in Appendix C.

4.4 Viruses

ASI's affiliated laboratory at the University of New Hampshire performed viral analyses for the two samples. The analyses evaluated the presence of human enteric viruses by integrated cell culture/nested polymerase chain reaction (ICC/nPCR), and coliphage by the EPA Method 1602 plaque assay procedure.

The coliphage assay found both male-specific and somatic coliphage in both samples (Table 8). Coliphages are viruses that infect *E. coli* bacteria, so their detection strongly indicates fecal contamination in the samples. The detection of these coliphages also indicated the possible presence of human enteric virus (ASI 2003c).

The ICC/nPCR procedure was optimized for the detection of eight types of enteric viruses, including adenovirus, astrovirus, enteroviruses (Coxsackie virus, Echovirus, and poliovirus), reovirus, rotavirus, and Hepatitis A virus (HAV) using BGMK, MA104, CaCo2, and FRhK cell lines. The analyses detected the presence of reovirus, a broad class of viruses. The analyses did not detect the presence of adenovirus, astrovirus, enteroviruses, HAV, and rotavirus.

The results of the virus analyses to date showed strong evidence of one or more infectious viruses present in both CSO01-SAY-092303 and CSO02-SAY-092303. These samples may contain other infectious viruses in addition to the identified reovirus.

The analytical evidence indicating the presence of one or more infectious viruses in the CSO01-SAY-092303 sample includes the following:

- Cytopathic effect (CPE) was observed after approximately 7 days during the first passage of the sample filtrate on BGMK (Buffalo Green Monkey

Kidney) cells, but was observed after only 48 hours during the second passage.

This strongly suggests that the infectious agent replicated in the BGMK cells during the first passage, since going from one passage to the next involves significant dilution (lysing the cells, adding cell culture broth, etc.). If the cellular toxicity effect was due to a chemical, one would expect its effect to be diluted out or perhaps be the same as before. In contrast, the CPE increased on the second passage, which suggests that it was the result of an increased concentration of infectious agent after being allowed to incubate/replicate in the appropriate cell host.

Koch's postulates to identify an infectious agent, as applied in microbiology, are that: 1) the agent must be isolated from one individual, 2) it must be purified and then put into a second individual, 3) it must cause the same symptoms in the second individual as in the first, and 4) it must be recoverable from the second individual as it was from the first (Last 1988). The CPE results of the analyses fulfill these postulates.

- As part of the initial screening and toxicity examination, an aliquot of sample was passed through a membrane filter with an absolute pore-size rating of 0.22 microns (μm). The detected infectious agent was in the filtrate, and therefore is concluded to be less than 0.22 μm in size (i.e., consistent with the sizes of virus particles).

In summary, the virus analyses showed:

- Male-specific and somatic coliphages were present in both samples, which indicates the presence of human feces and suggests the possible presence of human enteric virus(es).

- CSO01-SAY-092303 contained one or more infectious virus(es) based on CPE results and particle size. Reovirus was detected in this sample. Adenovirus, astrovirus, enteroviruses, HAV, and rotavirus were not detected.
- CSO02-SAY-092303 does appear to contain a reovirus. Adenovirus, astrovirus, enteroviruses, HAV, and rotavirus were not detected.

Laboratory data reports can be found in Appendix C.

4.5 Comparison of Bacteria Data to Interstate Environmental Commission Data

The Interstate Environmental Commission (IEC) conducted six rounds of pathogen sampling in the Passaic River between August 11 and September 22, 2003, at six sampling stations (IEC 2003). Figure 5 presents a map illustrating the IEC sampling locations. The IEC samples were analyzed for fecal coliform, total coliform, fecal *streptococcus*, and *enterococcus* for the 36 samples collected. Table 9 summarizes the IEC results.

The Exponent samples contained higher measured levels of pathogens than the IEC samples. This is expected, because the IEC samples were not collected immediately after a rainfall and were probably not collected near a discharging CSO pipe.

The IEC data showed that pathogen levels increased by more than 10-fold when rain had fallen in the prior 24 or 48 hours. This suggests that wet-weather events result in pathogen discharge into the Passaic River, and it is consistent with Exponent's samples, which showed a CSO pipe discharging pathogens at a level similar to raw sewage following a rainstorm.

The levels of fecal coliform and enterococcus were above the NJDEP's standards (NJDEP 1998) and the EPA's criterion (U.S. EPA 1986) in all but one sample. The water quality

standards and criterion were exceeded even when rain had not fallen in the prior 24 or 48 hours. This suggests that the ambient water quality in the Passaic River is poor with respect to pathogens, even without rainstorm-related CSO discharge into the river.

An evaluation of the National Climatic Data Center's (NCDC 2003a,b) 24-hour rainfall data prior to collection of the IEC samples indicates that the pathogen levels were still elevated well after rainfall ended. For example, elevated pathogen levels were measured in the August 11, 2003, sample collected at 10:40 a.m. The last recorded rainfall prior to collection of that sample was on August 10, 2003, between 2:00 and 3:00 p.m. This indicates that pathogen levels in the Passaic River can be elevated as much as 20 hours after rainfall.

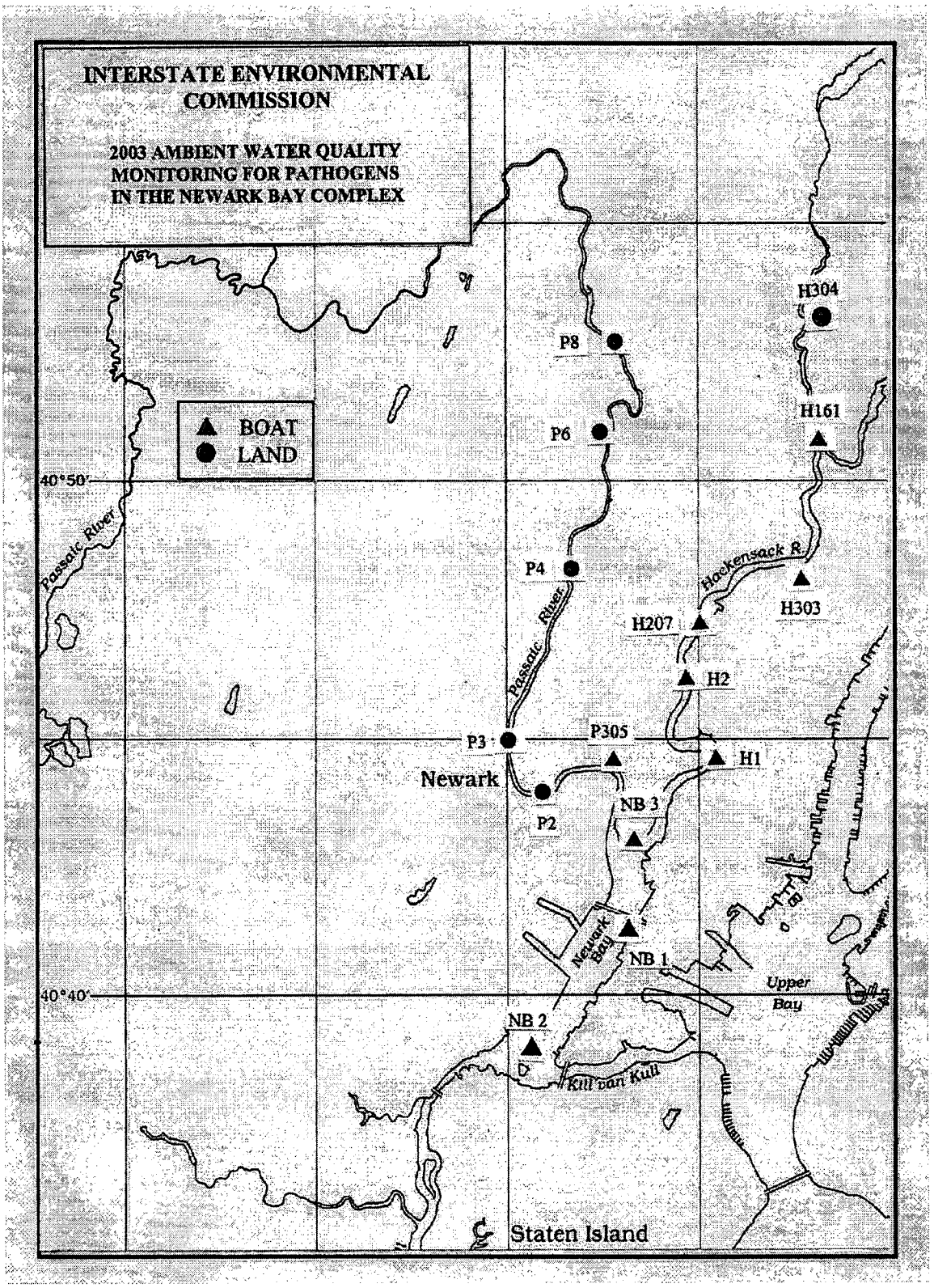

Figure 5. IEC pathogen sampling locations

Table 6. Results of bacteria analyses for sample CSO01-SAY-092303

Parameter	Result CSO01-SAY-092303	Result CSO02-SAY-092303	Notea
Total coliform	>30,000 CFUb/100 mL	>30,000 CFUb/100 mL	Total coliforms include several genera of bacteria that occur naturally in human and animal intestinal tracts, as well as in soils, fresh water, and marine waters. They are generally used as a rough estimate of water quality and as a screen for fecal coliforms.
Fecal coliform	>30,000 CFU/100 mL	>30,000 CFU/100 mL	Water quality indicator species. The result is well above the NJDEP (1998) water quality standard of 1,500 CFU/100 mL.
Fecal *streptococcus/enterococcus*	>30,000 CFU/100 mL	>30,000 CFU/100 mL	Fecal *streptococci/enterococci* are found in the gastrointestinal tract of warm-blooded animals. The result is well above the NJDEP (1998) water quality standard and the U.S. EPA (1986) criterion of 104 CFU/100 mL.
Escherichia coli	Positive	Positive	*E. coli* is a species of fecal coliform bacteria and is considered a direct indicator of fecal contamination from warm-blooded animals (U.S. EPA 2001; USGS 2000). EMSL Lab indicated that *E. coli* was clearly present, but it was not quantifiable because the other species were present in overwhelming numbers (EMSL 2003b).
Citrobacter freundii	3,000 CFU/mL	NF	These bacteria are commonly found in human stools. They can cause neonatal meningitis and are unique in their frequent association with brain abscess formation. The mortality and morbidity of Citrobacter meningitis is high (Badger et al. 1999).
Gram-negative rods resembling *Enterobacter* species	NF	4,000 CFU/mL	These gram-negative rods are widely distributed in nature, occurring in fresh water, soil, sewage, plants, vegetables, and animal and human feces. Several species may cause disease in humans with poor or somehow weakened immune systems.
Gram-variable rods resembling *Myroides odoratus*	NF	3,000 CFU/mL	*Myroides odoratus* and *Myroides odoratimimus* (formerly designated in a single species as *Flavobacterium odoratum*) are gram-negative aerobes and sources of nosocomial (health care-related) infections in humans, and disease in humans with poor or somehow weakened immune systems (Mammeri et al. 2002).

Table 6 (cont.)

Parameter	Result CSO01-SAY-092303	Result CSO02-SAY-092303	Notea
Rathayibacter/Rothia species	NF	10,000 CFU/mL	*Rathayibacter* are associated with plants, and some species may be pathogenic to livestock (Riley et al. 2003). *Rothia* is related to *Actinomycetes* and occur mainly in the oral cavity and on mucous membranes of warm-blooded vertebrates (Kalfas and Edwardsson 1990). They commonly cause dental infections in association with other bacteria. Both species were so closely together on the plate that they were grouped together (EMSL 2003b).
Aeromonas ichthiosmia	5,000 CFU/mL	NF	These bacteria occur in fresh water and sewage and are often found in feces of humans with diarrhea. Some species can cause gastroenteritis in children, wound infection, and less commonly, endocarditis, meningitis, pneumonia, osteomyelitis, peritonitis, conjunctivitis, thrombophlebitis, and chloecytitis (Murray et al. 1995).
Enterobacter cloacae	NF	2,000 CFU/mL	This species is associated with animal and human feces. It is of concern because it is one of the antibiotic-resistant bacteria and has been associated with pediatric blood infections in hospitals (Aiello et al. 2003; Selenic et al. 2003).
Pantoea dispersa	4,000 CFU/mL	NF	These bacteria are derived from plant surfaces, seeds, soil, and water, as well as from animals and human clinical specimens. They may cause disease in humans with poor or weakened immune system.
Kluyvera ascorbata	3,000 CFU/mL	NF	These gram-negative rods occur in food, soil, sewage, and human clinical specimens. They may cause disease in humans with poor or weakened immune systems.
Microbacterium lacticum	3,000 CFU/mL		This gram-positive rod is found in dairy products, sewage, and insects.
Actinomycetes	8,000 CFU/mL	10,000 CFU/mL	These slender gram-positive rods occur mainly in the oral cavity and on mucous membranes of warm-blooded vertebrates. They commonly cause infections in association with other bacteria.

Table 6 (cont.)

Parameter	Result CSO01-SAY-092303	Result CSO02-SAY-092303	Notea
Acinetobacter species	NF	3,000 CFU/mL	These gram-negative rods occur naturally in soil, water, and sewage.
Gram-variable rods	2,000 CFU/mL	1,000 CFU/mL	Other unidentified bacteria.
Gram-negative rods resembling *Pantoea* species	NF	2,000 CFU/mL	These gram-negative rods are isolated from plant surfaces, seeds, soil, and water, as well as from animals and human clinical specimens. They may cause disease in humans with poor or somehow weakened immune systems.
Gram-positive rods	NF	3,000 CFU/mL	Other unidentified bacteria that stain purple (positive with the gram stain

[a] Unless otherwise noted, the bacteria information were provided by EMSL (2003a).

[b] CFU – Colony-forming unit

[c] NF – not found

Table 7. Results of protozoans analyses for samples CSO01-SAY-092303 and CSO02-SAY-092303

Parameter	Sample		
	CSO01-SAY-092303	CSO02-SAY-092303	Note
Giardia	1.86×10^{3} cyst/L	7.98×10^{2} cyst/L	*Giardia* causes giardiasis, one of the most prevalent waterborne diseases in the United States;
Matrix spike recovery	11.2%	4.1%	
Cryptosporidium	<0.1 oocyst/L	<0.1 oocyst/L	*Cryptosporidium* causes cryptosporidiosis. Because infection with *Cryptosporidium* can occur after swallowing as few as 10–100 oocysts, swallowing a single mouthful of contaminated water from recreational swimming could cause illness (CDC 2000).
Matrix spike recovery	16.2%	8.1%	

Table 8. Results of coliphage analyses for samples CSO01-SAY-092303 and CSO02-SAY-092303

Parameter	Sample	
	CSO01-SAY-092303	CSO02-SAY-092303
Male-specific coliphage	2.3×10^{1} PFU/mL	3.4×10^{1} PFU/mL
Somatic coliphage	2.2×10^{1} PFU/mL	4.2×10^{1} PFU/mL

Table 9. Pathogen data from Interstate Environmental Commission (2003)

Date	Time	Station	Rainfall in Last 24 Hr (in.)	Rainfall in Last 48 Hr (in.)	Most Recent Precipitation Ended*	Fecal coliforms (MPN/100 mL)	Total coliforms (MPN/100 mL)	Fecal *Streptococcus* (MPN/100 mL)	*Enterococcus* (MPN/100 mL)
8/11/2003	10:40 AM	P2	0.73	1.32	8/10/03 3:00 PM	9,300	46,000	2,400	2,400
8/11/2003	10:55 AM	P3				46,000	110,000	4,600	4,600
8/11/2003	11:08 AM	P4				9,300	46,000	430	430
8/11/2003	11:20 AM	P6				2,300	4,300	930	930
8/11/2003	11:31 AM	P8				1,200	24,000	210	210
8/11/2003	5:35 PM	P305				9,300	15,000	750	750
8/12/2003	8:55 AM	P2	0.01	0.74	8/11/03 6:00 PM	930	24,000	1,500	390
8/12/2003	9:09 AM	P3				4,300	9,300	75	75
8/12/2003	9:19 AM	P4				4,300	15,000	430	430
8/12/2003	9:30 AM	P6				24,000	>240,000	>24,000	>24,000
8/12/2003	9:41 AM	P8				9,300	46,000	430	430
8/12/2003	10:07 AM	P305				4,300	9,300	430	430
8/18/2003	9:25 AM	P2	0.16	0.16	8/17/03 7:00 PM	7,500	46,000	930	930
8/18/2003	9:44 AM	P3				4,300	4,300	430	430
8/18/2003	9:50 AM	P4				4,300	4,300	430	230
8/18/2003	10:05 AM	P6				9,300	15,000	430	230
8/18/2003	10:15 AM	P8				4,300	4,300	43	23
8/18/2003	12:46 PM	P305				24,000	24,000	15	15
9/3/2003	8:42 AM	P2	0	0	9/4/03 11:00 AM	230	1,500	430	430
9/3/2003	8:56 AM	P3				230	2,300	2,400	2,400
9/3/2003	9:05 AM	P4				230	930	4,600	2,400
9/3/2003	9:20 AM	P6				9,300	9,300	2,400	2,400
9/3/2003	9:31 AM	P8				430	9,300	2,400	2,400
9/3/2003	1:11 PM	P305				430	1,500	43	43

Table 9 (cont.)

Date	Time	Station	Rainfall in Last 24 Hr (in.)	Rainfall in Last 48 Hr (in.)	Most Recent Precipitation Ended*	Fecal coliforms (MPN/100 mL)	Total coliforms (MPN/100 mL)	Fecal *Streptococcus* (MPN/100 mL)	*Enterococcus* (MPN/100 mL)
9/15/2003	9:16 AM	P2	0.04	0.53	9/14/03 4:00 AM	9,300	46,000	4,600	2,400
9/15/2003	9:30 AM	P3				15,000	46,000	11,000	11,000
9/15/2003	9:40 AM	P4				4,300	24,000	2,400	2,400
9/15/2003	9:53 AM	P6				2,300	24,000	4,600	2,400
9/15/2003	10:01 AM	P8				4,300	9,300	2,400	2,400
9/15/2003	11:10 AM	P305				3,900	46,000	4	<3
9/22/2003	9:16 AM	P2	0	0	9/19/03 6:00 AM	2,300	2,300	2,400	2,400
9/22/2003	9:27 AM	P3				430	2,300	2,400	2,400
9/22/2003	9:35 AM	P4				230	230	11,000	11,000
9/22/2003	9:45 AM	P6				2,300	7,500	11,000	11,000
9/22/2003	9:56 AM	P8				930	4,300	4,600	4,600
9/22/2003	10:15 AM	P305				2,300	4,300	430	430

* Data from NCDC (2003a,b).

5 Preliminary Human Health Risk Assessment

A preliminary human health risk assessment was conducted to evaluate the potential human health risks from exposure to pathogens in the surface waters of the lower Passaic River. The preliminary risk assessment describes the quantifiable risks associated with pathogens that have literature-reported mathematical exposure-response relationships and identifies hazards (non-quantifiable risks) for the pathogens associated with diseases but for which there are not literature-reported mathematical exposure-response relationships. The pathogen data used for this preliminary risk assessment are from the samples collected by Exponent on September 23, 2003.

5.1 Hazard Identification

The samples collected from two locations near the Saybrook Place CSO following the storm event were found to contain many pathogens. The levels of the pathogen indicator species total coliform, fecal coliform, fecal *streptococcus/enterococcus*, and the presence of *Escherichia coli* suggest that human exposures to the affected surface water are nearly equivalent to exposures to raw sewage, and therefore pose a potential health hazard. Human exposures to water bodies with elevated levels of these indicators have been associated with gastrointestinal illnesses (U.S. EPA 1986).

Fecal coliforms are a group of bacteria that are used as an indicator of sewage contamination. The New Jersey water quality criterion is 1,500 fecal coliforms per 100 mL of water for secondary-contact use and "any other reasonable use" of a saline estuary such as the lower Passaic River (NJDEP 1998). The fecal coliform levels in water from both sampling locations were more than 20 times higher than the NJDEP (1998) water quality standard for this portion of the lower Passaic River. The levels of fecal *streptococcus/enterococcus* in water from both sampling locations were more than 300 times higher than both the NJDEP (1998) water quality standard for this portion of the lower Passaic River and the U.S. EPA (1986) criterion. The

New Jersey water quality criteria document does not describe the health basis of its fecal coliform and fecal *streptococcus/enterococcus* standards. The NJDEP standards are identical to the EPA's criteria for designated beach areas; therefore, it is quite likely that the New Jersey standards are based on EPA criteria (U.S. EPA 1976, 1986). EPA has evaluated the mathematical relationship between fecal coliform and fecal *streptococcus/enterococcus* counts and reported swimming-related gastrointestinal illnesses. They found the illness rates associated with 200 fecal coliforms/100 mL and 104 fecal *streptococcus/enterococcus*/100 mL to be 8 illnesses per 1,000 swimmers at freshwater beaches, and 19 illnesses per 1,000 swimmers at marine beaches (U.S. EPA 1986).

The presence of several bacteria species and indicators clearly shows that human exposures to the affected surface waters will result in contact with bacterial pathogens associated with sewage. The health hazard of human contact with the surface water is significant, because several species are opportunistic pathogens that can cause infections in the young, the old, and those with compromised immune systems. The presence of *Citrobacter freundii* is significant, because it can cause neonatal meningitis, and it has been reported that the morbidity and mortality potentials of *Citrobacter* meningitis are high (Badger et al. 1999).

Giardia protozoan cysts were found in both samples at levels similar to those found in raw sewage. The human health effect of *Giardia* infection is clinically known as giardiasis, a diarrhea with loose, foul-smelling stools that are greasy, frothy, or bulky. Giardiasis is the most commonly reported intestinal protozoan infection worldwide, and *Giardia* is the most commonly identified parasite in the United States. The giardiasis patient suffers abdominal cramps, bloating, nausea, decreased appetite, malaise, and weight loss. In untreated individuals, the illness lasts about one week on average, and the infection lasts 6 weeks. Chronic diarrhea due to giardiasis can last an average of 1.9 years. Of *Giardia*-infected individuals, 0.5% will require hospitalization. Mortality associated with *Giardia* infections is rare (1×10^{-6}). Children are particularly susceptible to *Giardia* infection. Immunocompromised individuals may be at a greater risk of acquiring a chronic giardiasis infection with chronic diarrhea (U.S. EPA 1998). For some people, the giardiasis becomes a life-long illness.

The results of the virus analysis to date do not clearly indicate a virus-related hazard to human health. The presence of a virus that infects *E. coli*—male-specific and somatic coliphages—in both samples strongly indicates the presence of human feces in the CSO discharge and in the nearby water body. The presence of coliphages suggests a potential health hazard due to sewage and indicates that the condition is suitable for the presence of human virus. The coliphages are not, by themselves, associated with specific human diseases.

Reovirus was detected in the CSO discharge samples. Reovirus is a class of viruses that can infect both mammalian and non-mammalian species. The laboratory analysis of the sample was not able to discern whether the reovirus is the type that could infect humans. If the detected reovirus is infectious to humans, this virus is known to cause mild upper respiratory diseases and gastroenteritis. It is also known that reovirus is highly infectious, such that this virus has been modified and applied as one type of genetic therapy for human cancer. It is clear that the CSO discharge contains an infectious virus, but its identity is unclear at this time.

5.2 Exposure-Response Relationship Between Pathogen Levels and Illness

Exposure-response relationships for fecal coliform, fecal *streptococcus/enterococcus*, and *Giardia* that were quantified in the samples are available from the literature and are described below. There is no well-established exposure-response relationship for the remaining pathogens identified.

5.2.1 Fecal Coliform

For this risk assessment, the endpoint for fecal coliform exposure is swimming-related gastrointestinal illness, and the exposure-response relationship is based on the EPA's 1976 standard of 200 fecal coliforms/100 mL causing 19 illnesses per 1,000 swimmers (U.S. EPA 1976, 1986). The exposure-response relationship translates to a 9.5×10^{-5} gastrointestinal illness risk per one fecal coliform per 100 mL.

5.2.2 Fecal *Streptococcus/Enterococcus*

For this risk assessment, the endpoint for fecal coliform exposure is swimming-related gastrointestinal illness, and the exposure-response relationship is based on the EPA's 1986 standard of 104 fecal *streptococcus/enterococcus*/100 mL causing 19 illnesses per 1,000 swimmers (U.S. EPA 1986). In U.S. EPA (1986), the exposure-response relationship is described with the following mathematical equation:

$$\text{mean enterococci density} = \text{Antilog}_{10} \frac{(\text{illness rate/1000 people} - 0.20)}{12.17}$$

This equation can be used to solve for illness rates, as:

$$\text{Illness Rate/1000 people} = 12.17 \times \log_{10}(\text{mean enterococci density}) + 0.20$$

5.2.3 Giardia

For this preliminary risk assessment, the health endpoint is *Giardia* infection. The EPA's water quality criteria document for *Giardia* (U.S. EPA 1998) describes a human health risk assessment methodology using a dose-response model developed by Rose et al. (1991):

$$P = 1 - \exp(-rN).$$

The variables in this model are defined as:

P = Probability of infection

r = Fraction of organisms ingested that initiate infection

N = Average number of ingested organisms.

The value of r developed by Rose et al. (1991) was 0.01982 (95% CI 0.009798–0.03582). This dose-response model was based on the experimental data of Rendtorff (1954), wherein doses of *Giardia* ranging from 1 to 10^6 cysts were ingested by human volunteers (Rose et al. 1991).

5.3 Exposure Assessment

Three likely exposure scenarios were evaluated for this human health risk assessment. The exposure scenarios evaluated in this risk assessment are:

- *Recreator* — Recreational contact of surface water by boaters and rowing team
- *Visitor* — Contact of surface water by shoreline visitors
- *Homeless* — Contact of CSO and surface water by homeless individuals living along the shoreline.

These exposure scenarios contemplate reasonable uses of this water body. The levels of exposure to pathogens are influenced by several key factors: 1) contact rate and intensity, 2) dilution of pathogen concentration, and 3) environmental survivability of the pathogens (U.S. EPA 2001). In this preliminary risk assessment, it is conservatively assumed that:

- The contacts occur shortly after the rainstorm ends, such that the CSO may still be discharging into the river.
- Dilution of pathogen concentrations in the surface water is minimal, because the contacts occurred shortly after the rainstorm and there are many discharging CSOs along the lower Passaic River.

- The environmental conditions have *de minimus* effect on survivability of the pathogens in the surface water, because the contacts occurred shortly after the rainstorm.

5.3.1 Exposures to Fecal Coliform, Fecal *Streptococcus/Enterococcus*

The levels of fecal coliform and fecal *streptococcus/enterococcus* found in the water samples collected directly from the CSO pipe and roughly 10 ft downstream were both reported to be >30,000 CFU/100 mL. For the exposure assessment, it is not possible to employ the conventional exposure modeling approach, because the exposure-response relationship was based on epidemiological association of swimmer exposure and reported illnesses. The epidemiological association is non-specific on the exposure route(s) and contact rate(s) typically considered in exposure assessment. In this preliminary risk assessment, the exposure route(s) and contact rate(s) of the three exposure scenarios are assumed to be similar to the swimmers evaluated by U.S. EPA (1986).

5.3.2 Exposures to *Giardia*

For the exposure assessment, the exposure route was assumed to be ingestion, and the exposure parameters are summarized in Table 10.

Table 10. Assumptions for *Giardia* exposure assessment

Parameter	Value	Assumption
Exposure-Point Concentration (cyst/L)		
Recreator	798	Lower of the two samples
Visitor	1.33×10^{3}	Average of the two samples
Homeless	1.86×10^{3}	Higher of the two samples
Ingested Volume of Water (L)		
Recreator	0.1	U.S. EPA RAGS (1989)
Visitor	0.24	1 cup
Homeless	0.71	3 cups
Contact rate	Once	Single exposure

Based on these assumptions, the estimated amounts of ingested *Giardia* cysts are 80, 314, and 1,320 for the recreator, visitor, and homeless individual, respectively.

5.4 Risk Characterization

5.4.1 Numeric Risk Estimates for Exposures to Fecal Coliform

Using the exposure-response relationship of 9.5×10^{-5} gastrointestinal illness risk per one fecal coliform per 100 mL, and the assumed exposure levels of >30,000 CFU/100 mL fecal coliforms for all three scenarios, the preliminary risk estimate yielded a value of >2.9. This value suggests that, if an individual is exposed under any one of the three exposure scenarios, the exposure will result in a 100% probability of gastrointestinal illness. In other words, if 100 persons were exposed to fecal coliforms at the measured levels, every person would contract gastrointestinal illness.

The fecal coliform level of >30,000 CFU/100 mL can also be interpreted as resulting in a risk more than 150 times greater than the EPA's 1976 criterion (200 fecal coliforms/100 mL) or more than 20 times greater than NJDEP's acceptable criterion (1,500 fecal coliforms/100 mL).

5.4.2 Numeric Risk Estimates of Exposures to Fecal *Streptococcus/Enterococcus*

Using the exposure-response relationship from U.S. EPA (1986) for gastrointestinal illness risk and the assumed exposure levels of >30,000 CFU/100 mL fecal *streptococcus/enterococcus* for all three scenarios, the preliminary risk estimate yielded a value of >0.055. This value suggests that, if an individual is exposed under any one of the three exposure scenarios, the exposure will result in a 5.5% probability of gastrointestinal illness. In other words, if 100 persons were exposed to fecal coliforms at the measured levels, five to six persons would contract gastrointestinal illness.

The fecal *streptococcus/enterococcus* level of >30,000 CFU/100 mL may also be interpreted as resulting in a risk more than 300 times above the NJDEP standard and U.S. EPA (1986) criterion of 104 fecal *streptococci/enterococci* per 100 mL.

5.4.3 Numeric Risk Estimates of Exposures to *Giardia*

The estimated risks of *Giardia* infection for the three exposure scenarios by applying the Rose et al. (1991) model are summarized in Table 11.

Table 11. Estimated risk of *Giardia* infection

Exposure Scenario	Estimated Risk	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Recreator	0.79	0.54	0.94
Visitor	1.0	1.0	1.0
Homeless	1.0	1.0	1.0

The risk estimates suggest that, for every 100 recreators, there will be 54 to 94 individuals, and on average 79 individuals, infected with *Giardia*. For the visitors and homeless, every exposed individual will be infected.

The levels of *Giardia* infection risk may actually be higher than the above estimates, because the measured concentrations in water represented only 4% to 12% analytical recovery. Based on these recovery percentages, we estimate that the number of ingested *Giardia* cysts can be as high as 1,946, 4,268, and 11,790 for the recreator, visitor, and homeless, respectively, based on the same exposure assumptions. These higher estimates of ingested *Giardia* will result in estimated risks of 1.0 for all three scenarios, suggesting that every exposed individual will be infected.

5.4.4 Uncertainty of Risk Estimates

The primary uncertainty of the quantitative risks estimated for fecal coliform, fecal *streptococcus/enterococcus*, and *Giardia* exposures was the assumed exposures. The exposure-point concentrations were based on limited data (two samples) and do not well represent the entire stretch of the lower Passaic River. However, recent data from the Interstate Environmental Commission (IEC 2003) suggest that there is widespread fecal contamination in the river. The levels of *enterococcus* may not be well characterized based on the analytical findings of fecal *streptococcus*. The measured fecal *streptococcus* may not be 100% *enterococcus*, and the fecal *streptococcus* levels were conservatively assumed to be entirely enterococcus.

The basic assumption in the exposure assessment that human contact occurred shortly after a rainstorm is conservative. The levels of pathogen exposure are expected to become lower as more time passes after the CSO discharge. In the exposure assessment, it was also assumed that the recreator, visitor, and homeless individual are exposed to fecal coliforms in a manner similar to the EPA's derivation of the fecal coliform criterion where the exposure pathway and intensity were not clearly delineated. For the exposures to *Giardia*, conservative assumptions of the amount of water ingestion were made for the visitor and the homeless individual. The water ingestion rate for the recreator was based on a conservative Superfund risk assessment default assumption.

The uncertainty of the exposure-response relationships is minor relative to the estimated exposures, because the exposure-response relationships for fecal coliform and *Giardia* were based on epidemiological or human volunteer data.

5.4.5 Unquantified Risk of Exposure to Other Pathogens

The health risks from exposures to many of the measured or detected pathogens are not quantified. The levels of several of these bacteria species and indicators in the affected surface water are equivalent to levels in raw sewage. Therefore, human contact with the affected surface water poses a clear health hazard. The level of risk for contracting specific illnesses, though un-quantified, may be significant, because several species are opportunistic pathogens that affect especially the young, the old, and those with compromised immune systems. The presence of *Citrobacter freundii* is significant, because it can cause meningitis with high morbidity and mortality potentials.

The presence of reovirus does not clearly suggest potential human health risk, because it is unclear at present whether the virus is infectious to humans.

5.4.6 Comparison to Risks from Exposure to Dioxin through Crab Consumption

In 2002, the NJDEP presented estimated risks associated with exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) through the consumption of crabs from the lower Passaic River (NJDEP 2002). The NJDEP's estimated risk of cancer from consumption of crabs containing dioxin ranged from 0.005 to 1.157. The NJDEP risk assessment focused exclusively on risk posed by dioxin in crabs and failed to evaluate the risk posed by other chemicals and pathogens. To put the dioxin risk into perspective, this section describes a comparison of the pathogen data and risk presented herein with the dioxin data and risk described by NJDEP (2002).

The pathogen-related risk estimates presented herein are comparable to, if not greater than, the dioxin risk estimates from NJDEP. The dioxin risk estimates were based on conservative exposure assumptions of consuming from 5 crabs per week to 15 crabs per day over a person's entire 70-year lifetime. As will be discussed in the next section, chronic exposures are more likely to be mitigated over time for many reasons. Hence, the NJDEP's assumption of lifetime exposure via crab consumption is far more conservative than the exposure assumptions used for the pathogen risk estimates. The NJDEP's dioxin risk estimates were also based on an exposure-response relationship that was derived from a laboratory animal experiment, the relevance of which to human health is questionable. The exposure-response relationships for pathogens, on the other hand, were based on human data. Clearly, the pathogen risk assessment in this report is far less uncertain and represents a more realistic risk characterization for the current and future users of the lower Passaic River than the NJDEP's crab risk assessment.

5.4.7 Public Health Challenges of Pathogen Health Risk

The health risks from pathogen exposures evaluated in this preliminary risk assessment are based on a single, acute exposure. Health risks associated with acute exposures usually pose a more difficult environmental management challenge than risks due to long-term exposure, such as might occur with environmental contaminants. Risks from chronic chemical exposure can be mitigated over time by reducing exposure via planned institutional controls, lifestyle changes, or the normal periodic residential relocation or workplace changes that occur during a person's lifetime. Acute health risks, on the other hand, are difficult to mitigate, because they represent sporadic, unpredictable, and uncontrollable events, such as a rainstorm causing a combined sewer system's uncontrolled discharge into a water body.

The types of health effects that result from pathogen exposure usually have acute detrimental impacts on individuals, such as gastrointestinal illnesses and meningitis. As noted earlier, Giardiasis may also become a chronic illness. The severity of acute illnesses and the costs of medical treatment and productivity loss may seem less than those of certain chronic illnesses. However, from a public health management perspective, acute illnesses are more difficult to

address than chronic illnesses. Acute illnesses require immediate medical attention, and if such is not provided, the illness may result in more serious health effects leading to chronic disability or even death. Infections due to pathogen exposure also pose a serious challenge to health-care professionals to quickly identify or hypothesize the identity of the pathogen(s) in order to prescribe an effective medial treatment. Pathogen-related illness outbreaks involving large numbers of people can also strain the health-care system due simply to the unanticipated volume of medical attention required over a short period of time. Unlike most chemical agents, pathogen infection also poses an additional challenge to the health-care system, because infectious agents from patients can infect others, including the health-care providers.

5.4.8 Impact on Human Use of the Lower Passaic River

The State of New Jersey designated the stretch of the lower Passaic River from the Second River to the confluence with the Newark Bay for secondary-contact (e.g., recreational) use and "any other reasonable use" (NJDEP 1998). The levels of pathogen indicators measured in the surface water following a rainstorm exceeded the New Jersey water quality standards and the EPA criteria. The health risk of gastrointestinal illness and *Giardia* infection estimated in this preliminary risk assessment suggest that nearly any person who contacts the surface water will become ill. The data from IEC also indicated that the ambient pathogen levels in the Passaic River exceed the New Jersey water quality standards and the EPA criteria, even without rainfall occurring in the prior 24 or 48 hours, and that pathogen levels may remain elevated 20 hours after a rainfall. The discharge of pathogens into the lower Passaic River via the CSO system is inconsistent with the Federal Clean Water Act water body designation policy. U.S. EPA states:

> The second rule is simply a reflection of the CWA's "fishable/ swimmable" goal (protection and propagation of fish, shellfish, and wildlife and recreation in and on the water), as articulated in EPA's regulations, which say that these uses should be designated for all waters, unless it is demonstrated that it is impractical to meet them. Only in those cases where the "downgrading" process has been followed (see next slide) can these uses be excluded from the DUs for a waterbody (U.S. EPA 2004).

The NJDEP has indicated that intended uses of the Passaic River include, but are not limited to, secondary recreational contact and the maintenance and migration of fish populations. However, it is clear that the concentration of pathogens discharged by the Saybrook CSO, and the associated level of health risk posed by such, is a clear threat to the intended uses of this water body as defined by NJDEP.

6 Conclusion

The Saybrook Place CSO discharged high levels of microbial contaminants into the lower Passaic River during the rainstorm on September 23, 2003. The levels of fecal bacterial indicators in the affected water were well above the NJDEP water quality criteria. The discharge contained a host of bacteria species that are associated with human fecal matter and are known to cause illnesses, some of which are serious and may be fatal.

This preliminary human health risk assessment evaluated three exposure scenarios (recreator, visitor, and homeless person) and found that extremely high risks of gastrointestinal illness and *Giardia* infection are associated with exposure to lower Passaic River surface water that is affected by the CSO discharge. There also is evidence of infectious virus(es) having discharged from the CSO, but the significance of the virus(es) to human health risk is unclear at present. Data collected by the IEC during the same period as the samples collected by Exponent indicate that pathogen concentrations in the Passaic River are elevated and that this can occur even when rainfall amounts are minimal. The IEC data serve to confirm the observations by Exponent.

Pathogen-related health risks are more difficult to mitigate and manage than exposures to environmental agents, such as chemicals. The level of pathogen-related health risk associated with the lower Passaic River threatens the current and intended public use of this water body.

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Appendix A

Standard Operating Procedures

Saybrook Place Combined Sewer Outfall, Surface Water Pathogen Sampling: Standard Operating Procedure

1. Purpose

This Standard Operating Procedure (SOP) provides a general guideline for sampling surface waters near the Saybrook Place Combined Sewer Outfall (CSO) located along the Passaic River, New Jersey.

2. Scope

A site-specific surface water pathogen sampling procedure is described. This sampling procedure will provide samples appropriate for the bacterial and viral analytical objectives.

3. Equipment and Materials

In addition to the equipment and materials listed under Passaic River Surface Water Pathogen Sampling Sample Packaging and Handling Standard Operating Procedure, the following are required:

- Health and Safety Plan
- Waterproof field log book
- Sample chain-of-custody (COC) forms
- Cellular phone
- Dictating machine
- Camera
- Horiba U-10 Multiple Meter Sensor
- Raingear, such as ponchos
- Surgical gloves
- Hard hat
- U.S. Coast Guard–approved personal flotation device (PFD) (i.e., life-vest)
- Safety goggles/glasses
- Alcohol solution/swabs
- Potable water
- Rinse bottles
- 100-ft heavy duty rope
- Pre-sterilized 32-gallon plastic trash can with lid (one per sample)
- Pre-sterilized 5-gallon plastic bucket (one per sample)
- Pre-labeled sample bottles provided by laboratories
- Bagged ice

- Tape measure
- Milk crate.

4. Procedures and Guidelines

4.1 Pre-Labeling Sample Bottles

Pre-labeling of sample bottles is needed, because the wet-weather conditions under which the sampling effort will likely be conducted will make it difficult to generate sample labels on site. Sample seals and tags will be filled out as described in SOP GEN-01, *Field Documentation* and *Passaic River Surface Water Pathogen Sampling Sample Packaging and Handling Standard Operating Procedure,* with the exception of the sample date and time information.

4.2 Pre-Sterilization

The 5-gallon plastic buckets and the 32-gallon plastic trash cans will be pre-sterilized with a 10% bleach solution, followed by rinsing with sodium thiosulfate solution to remove the chlorine residues. The sterilized containers will be sealed in new plastic bags until they are used.

4.3 Sampling

1. Identify appropriate sample location.
2. Rinse the pre-sterilized 5-gallon bucket and the 32-gallon trash can with river water.
3. For each sample, collect surface water using the 5-gallon bucket to fill the 32-gallon trash can.
4. Keep the 32-gallon trash can lid closed at all times, except to pour in the water sample.
5. Shake the filled 32-gallon trash can to homogenize the content.
6. Fill sample containers:
   a. Three 100-mL samples, followed by
   b. 50 1-L samples.
7. Fill in date and time information on sample tags and seals.
8. Dispose of the used 5-gallon bucket and 32-gallon trash can in the refuse bag.
9. Dispose of the used surgical gloves in the refuse bag.
10. Rinse the Horiba U-10 Multiple Meter Sensor.

Passaic River Surface-Water Pathogen Sampling, Sample Packaging and Handling: Standard Operating Procedure

1. Purpose

This Standard Operating Procedure (SOP) document provides a general guideline for sample packaging and handling of surface-water samples collected near the Saybrook Place Combined Sewer Outfall (CSO) located along the Passaic River, New Jersey.

2. Scope

Specific requirements for sample packaging and shipping must be followed to ensure the proper transfer and documentation of environmental samples collected during field operations. Procedures for the careful and consistent transfer of samples from the field to the laboratory are outlined herein.

3. Equipment and Materials

Specific equipment or supplies necessary to properly pack and ship environmental samples include the following:

- Ice in doubled, sealable bags (e.g., Ziploc®), frozen Blue Ice®
- Sealable airtight bags (assorted sizes)
- Large plastic garbage bags
- Paper towels
- Coolers
- Bubble wrap
- Fiber-reinforced packing tape
- Duct tape
- Clear plastic packing tape
- Scissors
- Chain-of-custody seals
- "Fragile," "This End Up," or "Handle With Care" labels
- Mailing labels
- Airbills for overnight shipment
- Chain-of-custody record/sample analysis request forms.

4. Procedures and Guidelines

The logistics for sample packaging and shipping should be specifically tailored to each study. Depending on the logistics of the operation, field personnel may transport samples to the laboratory themselves or use an overnight courier service. If a courier service is used, then Exponent field personnel need to be aware of any potentially limiting factors to timely shipping (e.g., availability of overnight service and weekend deliveries to specific areas of the country, shipping regulations, "restricted articles" [e.g., dry ice, formalin]; see SOP HS-01) prior to shipping the samples. Federal Express service locations can be determined by calling 1-800-463-3339. United Parcel Service locations can be determined by calling 1-800-742-5877.

The following steps should be followed to ensure the proper transfer of samples from the field to the laboratories.

At the sample collection site:

1. Appropriately document all samples using a logbook (see SOP GEN-01), the required sample container identification (i.e., sample labels and sample tags), and a chain-of-custody record/sample analysis request (COC/SAR) form (example provided at the end of this document). Fill out the COC/SAR form as described in SOP GEN-02.

2. Make sure all applicable laboratory quality control sample designations have been made on the COC/SAR form. Samples that will be archived for possible future analysis should be clearly identified on the COC/SAR form by noting the following: "Do Not Analyze: Hold and archive for possible future analysis," because some laboratories interpret "archive" to mean continue holding the residual sample after analysis.

3. Clean the gross contamination from the outside of all dirty sample containers to remove any residual material that may lead to cross-contamination.

4. Store each sample container in an individual sealable clear plastic bag that allows the sample label (example provided at the end of this document) to be read. If the sample containers are large, they should also be sealed in clear plastic bags (i.e., not opaque garbage bags).

5. If the samples have a required storage temperature, place a sufficient amount of ice in the sample cooler to maintain the temperature inside the cooler (e.g., 4°C) throughout the sampling day.

At the sample processing area (immediately after sample collection):

1. If the samples have a required storage temperature, then the samples should be cooled to and maintained at that temperature prior to shipping. For example, a sufficient amount of ice must be present in each sample cooler to maintain the temperature inside the cooler at 4°C throughout the shipping time to the laboratory and until processing begins.

2. Be aware of holding-time requirements for project-specific analytes, and arrange the sample shipping schedule accordingly.

3. Samples will be placed in secure storage (i.e., locked room or vehicle), or will remain in the possession of Exponent sampling personnel, until they are shipped, to maintain sample integrity and fulfill chain-of-custody requirements.

4. Samples should be stored in the dark (e.g., coolers kept shut).

At the sample processing area (just prior to shipping):

1. Check sample containers against the COC/SAR form to ensure that all samples intended for shipment are accounted for.

2. Choose the appropriate-size cooler (or coolers) and make sure that the outside and inside of each cooler is clean of gross contamination. If the cooler has a drain on the outside at the bottom of the cooler, the drain should be capped and thoroughly taped shut with duct tape to prevent leakage.

3. The cooler should be lined with bubble wrap, and a large plastic bag should be opened and placed inside the cooler.

4. Individually wrap each glass container (which at the sample collection site had already been placed in an individual sealable plastic bag) in bubble wrap. Place the wrapped samples into the large plastic bag in the cooler, leaving sufficient room for ice to keep the samples cold (i.e., 4°C).

5. If the samples have a required storage temperature, add enough ice or Blue Ice® to keep the samples refrigerated during overnight shipping (i.e., 4°C). Always over-estimate the amount of ice that you think will be required. Ice should be enclosed in a sealable plastic bag and then placed in a second sealable plastic bag to prevent leakage. Avoid separating the samples from the ice with excess bubble wrap, because the bubble wrap will insulate the containers from the ice. After all samples and ice have been added to the cooler, use bubble wrap to fill any empty space to keep the samples from shifting during transport.

6. If temperature blanks have been provided by the testing laboratory, include one temperature blank in each sample cooler.

7. Sign, date, and include any tracking numbers provided by the shipper on the COC/SAR form. Remove the back copy of the original COC/SAR form and retain this copy for the project records.

8. Place the rest of the signed COC/SAR form in a sealable bag and tape the bag containing the form to the inside of the cooler lid. Each cooler should contain an individual COC/SAR form for the samples in each respective cooler. If time constraints affect sample shipping and it becomes necessary to combine all of the samples onto a single set of COC/SAR forms for a

shipment that contains multiple coolers, indicate on the outside of the respective cooler "Chain-of-Custody Inside."

9. After the cooler is sufficiently packed to prevent shifting of the containers, close the lid and seal it shut with fiber-reinforced packing tape. The cooler should be taped shut around the opening between the lid and the bottom of the cooler and around the circumference of the cooler at both hinges.

10. As security against unauthorized handling of the samples, apply two chain-of-custody seals across the opening of the cooler lid (example provided at the end of this document). One seal should be placed on the front of the cooler, and one seal should be placed on the side of the cooler opposite the first seal. Be sure the seals are properly affixed to the cooler so they are not removed during shipment. Additional tape across the seal may be necessary if the outside of the cooler is wet.

11. Use a mailing label, label the cooler with destination and return addresses, and add other appropriate stickers, such as "This End Up," "Fragile," and "Handle With Care." If the shipment contains multiple coolers, indicate on the mailing label the number of coolers that the testing laboratory should expect to receive (e.g., 1 of 2; 2 of 2). Place clear tape over the mailing label to firmly affix it to the outside of the cooler and to protect it from the weather. This is a secondary label in case the airbill is lost during shipment.

12. If an overnight courier is used, fill out the airbill as required and fasten it to either the top of the cooler or to handle tags provided by the shipper. In addition to the adhesive backing on many airbills, the airbill and/or mailing label should also be taped to the lid, because tracking problems can occur if a sticker is removed during shipment.

13. Notify the laboratory contact and the Exponent project QA/QC coordinator that samples will be shipped, and the estimated arrival date and time. All environmental samples that are shipped at 4°C or -20°C will be shipped overnight for delivery the next morning. Fax copies of all COC/SAR forms to the Exponent QA/QC coordinator.

    Note: Prior to faxing, it may be necessary to photocopy the COC/SAR form on a slightly darker setting so that the form is readable after it has been faxed. Never leave the original COC/SAR form in the custody of non-Exponent staff.

CHAIN OF CUSTODY RECORD/SAMPLE ANALYSIS REQUEST FORM

Page ____ of ____

Project:
(Name and Number)

Exponent

Exponent Contact: _____ Office: ____

Ship to: _____

_____

_____

Lab Contact/Phone: _____

Samplers:

Environmental Group

Bellevue, WA (425) 643-9803
Boulder, CO (303) 444-7270
Lake Oswego, OR (503) 636-4338
Los Angeles, CA (310) 823-2035
Natick, MA (508) 652-8500

Sample No.	Tag No.	Date	Time	Matrix	Analyses Requested						Extra Container	Archive	Remarks

Matrix Code: GW - Groundwater SL - Soil SD - Sediment SW - Surface water

OTHER - Please identify codes _____

Priority:
☐ Normal ☐ Rush Rush time period _____

Shipped via: ☐ FedEx/UPS ☐ Courier Other _____

Condition of Samples Upon Receipt: _____

Custody Seal Intact: ☐ Yes ☐ No ☐ None

Relinquished by: _____ (Signature) Date/Time: _____ Received by: _____ (Signature) Date/Time: _____

Relinquished by: _____ (Signature) Date/Time: _____ Received by: _____ (Signature) Date/Time: _____

Distribution: White and Yellow Copies - Accompany Shipment; Pink Copy - Project File

Chain-of-custody/sample analysis request form.

Exponent

OFFICIAL SAMPLE SEAL

SAMPLE NO.	DATE
SIGNATURE	
PRINT NAME AND TITLE	

Exponent

SAMPLE NO.

SITE NAME

DATE TIME

SAMPLER PRESERVATIVE

TAG NO. 25101

Example label and custody seal.

Exponent

SOP GEN-01
FIELD DOCUMENTATION

The integrity of each sample from the time of collection to the point of data reporting must be maintained throughout the study. Proper record keeping will be implemented in the field to allow samples to be traced from collection to final disposition. All information relevant to field operations must be properly documented to ensure that activities are accounted for and can be reconstructed from written records. Several types of field documents and sample tracking information will be used for this purpose and should be consistently used by field personnel.

FIELD LOGBOOKS

During field sampling events, field logbooks are used to record all daily field activities. The purpose of the field logbook is to document events that occur and record data measured in the field to the extent that someone not present at the site can reconstruct the activity without relying on the memory of the field crew.

A bound, waterproof field logbook with consecutively numbered pages will be completed using indelible ink for each sampling event. All daily field activities will be documented in indelible ink in this logbook and no erasures will be made. All corrections should consist of a single line-out deletion, followed by the sampler's initials and the date. The sampler will initial and date each page of the field logbook. The sampler will sign and date the last page at the end of each day, and a line will be drawn through the remainder of the page.

The project name, site name and location (city and state), Exponent contract number, and the dates (i.e., duration) of sampling activity should be written on the cover of the field logbook. If more than one logbook is used during a single sampling event, then the upper right hand corner of the logbook will be annotated (e.g., 1 of 2, 2 of 2) to indicate the number of logbooks used during the field event.

Field logbooks will be stored in a secure manner when not in use in the field. At a minimum, the sampler will record the following information in the field logbook:

- Project name, project location, and project number
- Purpose and description of the field task
- Project start date and end date
- Date and time of entry (24-hour clock)

- Time and duration of daily sampling activities
- Weather conditions at the beginning of the field work and any changes that occur throughout the day, including the approximate time of the change (e.g., wind speed and direction, wave action, current, tide, vessel traffic, temperature of both the air and water, thickness of ice if present)
- Name of person making entries and other field personnel and their duties, including the times that they are present
- Level of personal protection being used
- Onsite visitors, if any, including the times that they are present
- The name, agency, and telephone number of any field contacts
- Notation of the system used to determine the station location information
- The sample identifier and analysis code for each sample to be submitted for laboratory analysis
- All field measurements made (or reference to specific field data sheets used for this purpose), including the time that the measurement was collected and the date of calibration, if appropriate
- The sampling location name, date, gear, water depth (if applicable), and sampling location coordinates
- The type of vessel used (e.g., size, power, type of engine) (for aquatic sampling only)
- The location and description of the work area, including sketches and map references, if appropriate
- Specific information on each type of sampling activity
- The sample type (i.e., groundwater, soil, surface sediment), sample number, and sample tag number
- Preservatives used, if any
- Sample storage methods
- Cross-references of numbers for duplicate samples
- A description of the sample (source and appearance, such as soil or sediment type, color, texture, consistency, presence of biota or debris, presence of oily sheen, changes in sample characteristics with depth, presence/location/ thickness of the redox potential discontinuity (RPD) layer, and odor) and penetration depth

- Estimate of length and appearance of recovered cores
- Photographs (uniquely identified) taken at the sampling location, if any
- Variations, if any, from specified sampling protocols and reasons for deviation
- Details pertaining to unusual events which might have occurred during sample collection (e.g., possible sources of sample contamination, equipment failure, unusual appearance of sample integrity, control of vertical descent of the sampling equipment)
- References to other logbooks used to record information (e.g., field data sheets, health and safety log).
- The signature of the person making the entry.

Upon completion of the field sampling event, the field team leader will be responsible for submitting all field logbooks to be copied. A discussion of copy distribution is provided below.

FIELD DATA FORMS

Occasionally, additional field data forms are generated during a field sampling event (e.g., Station/Sample Log, Groundwater Monitoring Form, Sediment Core Profile Form) to record the relevant sample information collected during a sampling event. For instructions regarding the proper identification of field data forms, sampling personnel should consult the project-specific field sampling plan.

Upon completion of the field sampling event, the field team leader will be responsible for submitting all field data forms to be copied. A discussion of copy distribution is provided below.

PHOTOGRAPHS

In certain instances, photographs (print or digital) of sampling stations will be taken using a camera-lens system with a perspective similar to the naked eye. Photographs may also be taken of sample characteristics and routine sampling activities. Photographs should include a measured scale in the picture, when practical. Telephoto or wide-angle shots will not be used because they cannot be used in enforcement proceedings. The following items should be recorded in the field logbook for each photograph taken:

1. The photographer's name, the date, the time of the photograph, and the general direction faced
2. A brief description of the subject and the field work portrayed in the picture

3. The sequential number of the photograph (filename for digital) and the roll number (disk number for digital, if applicable) on which it is contained.

Upon completion of the field sampling event, the field team leader will be responsible for submitting all photographic materials to be developed (slides, prints) or to be copied (disks), as appropriate. The slides, prints, or disks (as appropriate) and associated negatives will be placed in the project files (at the Exponent Project Manager's location [project-specific]). Photo logs and any supporting documentation from the field logbooks will be photocopied and placed in the project files with the slides, prints, or disks.

SAMPLE LABELS

Exponent sample labels are designed to uniquely identify each sample container that is collected during a sampling event. Field crews will be provided with preprinted sample labels, which must be affixed to each sample container used. The labels should be filled out at the time the samples are collected and should consist of the following information:

1. Sample number
2. Site name or project number
3. Date and time sample is collected
4. Initials of the samplers
5. Preservatives used, if any
6. A unique number (commonly referred to as the "Tag Number") that is preprinted on the label consisting of six digits; used to identify individual containers.

SAMPLE TAGS

Exponent sample tags are designed to be affixed to each container that is used for a sample. Sample tags are only required for environmental samples collected in U.S. Environmental Protection Agency (EPA) Region 5. Field crews will be provided with preprinted sample tags. Sample tags must be attached to each individual sample container with a rubber band or wire through a reinforced hole in the tag. All sample tag entries will be made with indelible ink. The tags should be filled out at the time the samples are collected and should consist of the following information:

1. Sample number
2. Site name or project number

3. Date and time sample is collected
4. Initials of the samplers
5. Preservatives used, if any
6. Type of analysis.

A space for the laboratory sample number (provided by the laboratory at log-in) will also be provided on the sample tag.

INTERNAL SAMPLE LABELS

For benthic infaunal samples, the sediment is washed away from the sample and the remaining benthic infauna are collected into a sample container. A sample label as discussed above is affixed to the outside of the sample container. In addition, an internal sample label is placed inside the sample container. This internal sample label is made of water-proof paper and all internal sample label entries will be made with pencil. The internal sample labels should be filled out at the time the samples are collected and should consist of the following information:

1. Sample number
2. Site name or project number
3. Date and time sample is collected
4. Initials of the samplers
5. Preservative used (i.e., formalin).

CHAIN-OF-CUSTODY/SAMPLE ANALYSIS REQUEST FORMS

Exponent uses a combined chain-of-custody/sample analysis request (COC/SAR) form. The sample number and the unique number at the bottom of each sample label will be recorded on the COC/SAR form. The COC/SAR form will also identify the sample collection date and time, the type of sample, the project, and the field team leader. In addition, the COC/SAR form provides information on the preservative or other sample pretreatment applied in the field and the analyses to be conducted by referencing a list of specific analyses or the statement of work for the laboratory. The COC/SAR form will be sent to the laboratory along with the sample(s).

The COC/SAR form will be completed in triplicate and consists of three pages: a white sheet, which always remains with the samples; a yellow sheet, which remains with the samples when they are shipped to the laboratory; and a pink sheet, which is removed by field staff prior to shipping to the laboratory or prior to placing the samples into the sample archives. The white sheet and the yellow sheet will be placed into a plastic sealable bag and secured to the inside top

of each sample cooler. The pink sheet will be retained by the field staff for filing at the Exponent Project Manager's location (project-specific).

Exponent also uses computer-generated COC/SAR forms. If computer-generated forms are used, then the forms must be printed in triplicate and all three sheets signed so that two sheets can accompany the shipment to the laboratory and one sheet can be retained on file at the Exponent Project Manager's location (project-specific).

At the end of each sampling day and prior to shipping or storage, chain-of-custody entries will be made for all samples. Information on the labels and tags will be checked against filed logbook entries. Upon completion of the field sampling event, the field team leader will be responsible for submitting all COC/SAR forms to be copied. A discussion of copy distribution is provided below.

CUSTODY SEAL

As security against unauthorized handling of the samples during shipping, two custody seals will be affixed to each sample cooler (example provided in Attachment GEN-03-1). The custody seals will be placed across the opening of the cooler (front right and back left) prior to shipping. Be sure the seals are properly affixed to the cooler so they cannot be removed during shipping. Additional tape across the seal may be prudent.

SHIPPING AIRBILLS

When samples are shipped from the field to the testing laboratory via a commercial carrier (e.g., Federal Express, UPS), an airbill or receipt is provided by the shipper. Upon completion of the field sampling event, the field team leader will be responsible for submitting the sender's copy of all shipping airbills to be copied. A discussion of copy distribution is provided below. The airbill number (or tracking number) should be noted on the applicable COC/SAR forms or alternatively the applicable COC/SAR form number should be noted on the airbill to enable the tracking of samples if a cooler becomes lost.

ACKNOWLEDGMENT OF SAMPLE RECEIPT FORMS

In most cases, when samples are sent to a testing laboratory, an Acknowledgment of Sample Receipt form is faxed to the Exponent QA/QC coordinator the day the samples are received by the laboratory. It is the responsibility of the person receiving this form to review the form and make sure that all the samples that were sent to the laboratory were received by the laboratory and that the correct analyses were requested. If an error is found, the laboratory must be called immediately. Decisions made during the telephone conversation should be documented in writing on the Acknowledgment of Sample Receipt Form. In addition, corrections should be made to the COC/SAR form and the corrected version of the COC/SAR form should be faxed to the laboratory.

The Acknowledgment of Sample Receipt form (and any modified COC/SAR forms) will then be submitted to be copied. A discussion of copy distribution is provided below.

ARCHIVE RECORD FORMS

On rare occasions, samples are archived at an Exponent office. If samples are to be archived at Exponent, it is the responsibility of the project manager to complete an Archive Record form. This form is to be accompanied by a copy of the COC/SAR form for the samples, and will be placed in a locked file cabinet.

DISTRIBUTION OF COPIES

Two copies of all field logbooks, additional field data forms, COC/SAR forms, and Acknowledgement of Sample Receipt forms will be made at Exponent. The first copy will be stamped with a "COPY" stamp. This copy will be placed in the project file and will be available for general staff use. The second copy will be stamped with a "FILE" stamp. This copy will be placed in the data management file with the laboratory data packages and will be used by the data management and quality assurance staff only. The original field logbooks and forms will be placed in a locked file cabinet.

One copy of the shipping airbill will be made and placed in the project file. The original airbill will be given to the respective Exponent receptionist for filing and billing purposes.

Setup of Locking File Cabinet

Each project will have its own file folder in a locking file cabinet. The folder label will include the project name and charge number. As many as five kinds of files will be included in this folder for each project:

- Field logbook(s)
- Additional field data forms
- COC/SAR forms
- Acknowledgment of Sample Receipt forms
- Archive Record form (to be completed only if samples are archived at the Bellevue field storage facility or at the Boulder laboratory).

Note: SOPs GEN-01 and -03 cited within.

Exponent

SOP GEN-02
SAMPLE CUSTODY

A stringent, established program of sample chain-of-custody will be followed during sample storage and shipping activities to account for each sample. The procedure outlined herein will be used with SOP GEN-01, *Field Documentation*, and SOP GEN-03, *Sample Packaging and Shipping*. Chain-of-custody record/sample analysis request (COC/SAR) forms (Attachment GEN-03-1) ensure that samples are traceable from the time of collection through processing and analysis until final disposition. A sample is considered to be in a person's custody if any of the following criteria are met:

1. The sample is in the person's possession
2. The sample is in the person's view after being in possession
3. The sample is in the person's possession and is being transferred to a designated secure area
4. The sample has been locked up to prevent tampering after it was in the person's possession.

At no time is it acceptable for samples to be outside of Exponent personnel's custody unless the samples have been transferred to a secure area (i.e., locked up). If the samples cannot be placed in a secure area, then an Exponent field team member must physically remain with the samples (e.g., at lunch time one team member must remain with the samples).

PROCEDURE

The chain-of-custody record portion of the COC/SAR form is the most critical because it documents sample possession from the time of collection through the final disposition of the sample. The sample analysis request portion of the form provides information to the laboratory regarding what analyses are to be performed on the samples that are shipped.

The COC/SAR form will be completed after each field collection activity and before the samples are shipped to the laboratory. Sampling personnel are responsible for the care and custody of the samples until they are shipped. When transferring possession of the samples, the individuals relinquishing and receiving the samples must sign the COC/SAR form(s), indicating the time and date that the transfer occurs. Copies of the forms will be made and kept by Exponent, and the originals will be included with the samples in the sample cooler. The following guidelines will be followed to ensure consistent shipping procedures and to maintain the integrity of the samples:

1. Each chain-of-custody record/sample analysis request form must be appropriately signed by the sampling personnel. The person who relinquishes custody of the samples must also sign this form.

2. The chain-of-custody record/sample analysis request form should not be signed until the information has been checked for inaccuracies by the field team leader. All changes should be made by drawing a single line through the incorrect entry and initialing and dating it. Revised entries should be made in the space below the entries. Any blank lines remaining on the COC/SAR form after corrections are made should be marked out with single lines. This procedure will preclude any unauthorized additions.

3. At the bottom of each COC/SAR form is a space for the signatures of the persons relinquishing and receiving the samples and the time and date that the transfer occurred. The time that the samples were relinquished should match exactly the time they were received by another party. Under no circumstances should there be any time when custody of the samples is undocumented.

4. If samples are sent by a commercial carrier not affiliated with the laboratory, such as Federal Express or UPS, the name of the carrier should be entered in the "received by" block. Any tracking numbers supplied by the carrier should be also entered in the "received by" block. The time of transfer should be as close to the actual drop-off time as possible. After the COC/SAR forms are signed and copied, they should be sealed inside the transfer container.

5. If errors are found after the shipment has left the custody of Exponent personnel, a corrected version of the forms must be made and sent to all relevant parties. Minor errors can be rectified by making the change on a copy of the original with a brief explanation and signature. Errors in the signature block may require a letter of explanation.

6. Samples that are archived internally at Exponent must be accompanied by a COC/SAR form and an Archive Record form (see SOP GEN-01).

Appendix B

Field Notes

Weems & Plath

WetLog™

WATERPROOF LOGBOOK

Weems & Plath
214 Eastern Avenue
Annapolis MD 21403
410-263-8700/Fax: 410-263-8718
www.weems-plath.com

Cat. No. 801

9/22/03

11:30 AM Arrival on Beach

12:00 pm can located

Flow visible

Sand, flushing

3' above water line

1/2 of pipe submerged

water flow

0.7 pipe

0.4 from to pipe out other side of platform

12:15 First sample out pipe

LOG
GBOOK

One 100ml sample.
5x10L samples

12:30 pm Second sample at platform. Rain ended but CSO continued to discharge. One 100ml sample 5x10L samples

1:30pm Have all samples put in lab container

Physical Params

Platform - SAY02
pH - 7.46 Temp - 21.6°C
Cond - 0.30 mS/cm
Turb - 81 NTU Sal: 0.01 %
O_2 - 0.15 mg/L
Weather - Overcast no water

SAY01 CSO pipe
pH - 7.41
cond: 0.327 mS/cm
Turb = 80 NTU
O_2 = 0.38 mg/L
Temp - 22.1 °C
Sal : 0.01 %

SAY01	SAY02	TAG 20#
63147	63155	
63159	63157	
63151	63156	10L
63150	63161	
62160	63158	
63147	63153	100mL

2:36pm Depart Nanaimo.

Appendix C

Laboratory Data

Analytical Services, Inc.

P.O. Box 515
130 Allen Brook Lane
Williston, VT 05495

Phone: (802) 878-5138
Toll Free: (800) 723-4432
Fax: (802) 878-6765

February 20, 2004

Mark Harris, Ph.D.
Exponent, Inc.
10899 Kinghurst Dr., Suite 245
Houston, TX 77099
281-983-4016

Dear Mark,

As requested, enclosed are true and accurate copies of the Passaic River Project original data from the US EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (April 2001) analysis. Please note that, as discussed, Method 1623 was modified to include the use of ColorSeed™ (BTF Sydney, AU) as Internal Positive Controls. Included are the analytical report, the associated chain-of-custody and shipping documents, internal worksheets, bench sheets and method required laboratory ongoing precision and recovery documents.

Please contact me if you have any questions or require additional information. I may be reached at (800) 723-4432 x-15 or vial email at pwarden@analyticalservices.com.

Sincerely,

ANALYTICAL SERVICES. INC.

Paul S. Warden
Vice President

Analytical Services, Inc.

P.O. Box 515
130 Allen Brook Lane
Williston, VT 05495

Phone: (802) 878-5138
Toll Free: (800) 723-4432
Fax: (802) 878-6765

October 9, 2003

Mark Harris
Exponent, Inc.
10899 Kinghurst Drive, Suite 245
Houston, TX 77099

Dear Mark:

Enclosed please find the results of the USEPA Method 1623 analyses for *Giardia* and *Cryptosporidium* performed on the two (2) samples received in our laboratory on September 25, 2003.

Please note that the *Giardia* recovery efficiency was 11.2% for sample 63147 and 4.1% for sample 63161, both of which are outside of the established acceptance criteria of 15 – 118%. The *Cryptosporidium* recovery efficiency of sample 63161 was 8.1%, which is outside of the established acceptance criteria of 13 – 143%.

Thank you for using Analytical Services, Inc. for your testing needs. If you have any questions or if we may be of service in the future, please do not hesitate to contact Client Services at (800) 723-4432.

Sincerely,

ANALYTICAL SERVICES, INC.

Corey Fregeau
Staff Microbiologist

CF/lll

Project No.: 2003-0925-014

Analytical Services, Inc.

Client:	Exponent, Inc.	Report Date:	October 9, 2003
Address:	10899 Kinghurst Drive, Suite 245	Sample Receipt Date:	September 25, 2003
	Houston, TX 77099	Analyst:	cjf

USEPA Method 1623[1]
Giardia and *Cryptosporidium*
with Internal Positive Control (IPC)[2]
Analytical Report

ASI Sample ID No.: 2003-0925-014

Quality Control Batch No.: 236-1
Method Blank Laboratory No.: 2003-0925-013
Ongoing Precision and Recovery Laboratory No.: 2003-0925-012

Sample Information

Client Sample ID:	63147
Collection Date:	September 23, 2003
Collection Time:	1215 hrs
Matrix:	raw surface water
Sample Turbidity (NTU):	unknown
Sample Type[2]:	Field Sample with IPC (ColorSeed)

Volume and Filtration Information

Filter Type:	Gelman HV
Number of Filters Used:	2
Volume Spiked (L):	10.0
Volume Filtered (L):	10.0*
Volume Examined (L):	10.0**

Analytical Results

ANALYTE	INDIGENOUS (OO)CYSTS / LITER	COLORSEED SPIKE DOSE / LITER	COLORSEED (OO)CYSTS / LITER	COLORSEED RECOVERY
Giardia	1.86×10^3	9.8	1.1	11.2%
Cryptosporidium	0	9.9	1.6	16.2%

[1] **Method:** Samples processed, stained and examined using USEPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration, Immunomagnetic separation (IMS), and Immunofluorescence Assay (IFA) Microscopy (USEPA), modified to include IPC's (see below).

[2] **Note:** This sample was analyzed using ColorSeed™ (BTF, Australia), which is a gamma irradiated, flow cytometer counted IPC for *Cryptosporidium* and *Giardia*. IPC's are added directly to the sample, which is then concentrated, purified, stained and examined according to EPA Method 1623 (as applicable). The sample is examined for FITC-stained cysts and/or oocysts, and each one is examined under standard fluorescent filters and also red-fluorescence conditions. Indigenous (oo)cysts are counted and reported as per Method 1623. In addition, ColorSeed™ (oo)cysts are counted and recovery percentage is calculated, which allows determination of the efficiency of protozoa recovery from this sample matrix.

***Note:** 5.0 liters were filtered through each filter.

****Note:** Seven (7) aliquots were examined using IMS and IFA: five (5) aliquots of 1.0 liter each from the first filtration, and two (2) aliquots of 2.5 liters each from the second filtration.

Project No.: 2003-0925-014

Analytical Services, Inc.

Client: Exponent, Inc.
Address: 10899 Kinghurst Drive, Suite 245
Houston, TX 77099

Report Date: October 9, 2003
Sample Receipt Date: September 25, 2003
Analyst: cjf

USEPA Method 1623[1]
Giardia and *Cryptosporidium*
with Internal Positive Control (IPC)[2]
Analytical Report

ASI Sample ID No.: 2003-0925-015

Quality Control Batch No.: 236-1
Method Blank Laboratory No.: 2003-0925-013
Ongoing Precision and Recovery Laboratory No.: 2003-0925-012

Sample Information

Client Sample ID:	63161
Collection Date:	September 23, 2003
Collection Time:	1230 hrs
Matrix:	raw surface water
Sample Turbidity (NTU):	unknown
Sample Type[2]:	Field Sample with IPC (ColorSeed)

Volume and Filtration Information

Filter Type:	Gelman HV
Number of Filters Used:	1
Volume Spiked (L):	10.0
Volume Filtered (L):	10.0
Volume Examined (L):	10.0*

Analytical Results

ANALYTE	INDIGENOUS (OO)CYSTS / LITER	COLORSEED SPIKE DOSE / LITER	COLORSEED (OO)CYSTS / LITER	COLORSEED RECOVERY
Giardia	7.98×10^2	9.8	0.4	4.1%
Cryptosporidium	0	9.9	0.8	8.1%

[1] **Method:** Samples processed, stained and examined using USEPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration, Immunomagnetic separation (IMS), and Immunofluorescence Assay (IFA) Microscopy (USEPA), modified to include IPC's (see below).

[2] **Note:** This sample was analyzed using ColorSeed™ (BTF, Australia), which is a gamma irradiated, flow cytometer counted IPC for *Cryptosporidium* and *Giardia*. IPC's are added directly to the sample, which is then concentrated, purified, stained and examined according to EPA Method 1623 (as applicable). The sample is examined for FITC-stained cysts and/or oocysts, and each one is examined under standard fluorescent filters and also red-fluorescence conditions. Indigenous (oo)cysts are counted and reported as per Method 1623. In addition, ColorSeed™ (oo)cysts are counted and recovery percentage is calculated, which allows determination of the efficiency of protozoa recovery from this sample matrix.

***Note:** Two (2) aliquots of 5.0 liters each were examined using IMS and IFA.

Project No.: 2003-0925-014

From: Steave Su <ssu@exponent.com>
Subject: **Error in Exponent CoC for 9/23/03 samples**
Date: March 3, 2004 10:30:50 AM EST
To: Mark Harris <MHarris@exponent.com>

There is an error on the CoC form to Analytical Services (ASI) for the 9/23/03 CSO samples.
On the form both samples were identified as "CSO01-SAY-092303."
Based on my record (the log book) and the timing of the samples (12:15 vs 12:30 PM) the correct samples numbers are:

CSO01-SAY-092303 for Tag No. 63147
CSO02-SAY-092303 for Tag No. 63161

I have notified ASI of this error.

Steave H. Su, M.P.H.
Senior Scientist
Exponent
420 Lexington Avenue
Suite 408
New York, NY 10170
Tel: 212-972-9477
Fax: 212-972-9480
Cell: 973-879-9866
email: ssu@exponent.com

CHAIN OF CUSTODY RECORD/SAMPLE ANALYSIS REQUEST FORM

Page 1 of 1

Project: (Name and Number) PASSAIC RIVER NONRI/FS 8601068.005.0106

Exponent Contact: STEVE SU Office: AM

Ship to: Analytical Services Inc
130 Allen Brook Lane
Williston, VT 05495

Lab Contact/Phone: Jamie Burke 800-423-4432

Samplers: DAN GARDNER

Analyses Requested

Sample No.	Tag No.	Date	Time	Matrix	ERA Method 1623						Extra Container	Archive	Remarks
CS001-SAY-092303	63147	9/23/03	12:45PM	SW	✓								
CS001-SAY-092303	63161	9/23/03	12:30PM	SW	✓								

Exponent Environmental Group
Bellevue, WA (425) 643-9803
Boston, MA (781) 486-6681
Boulder, CO (303) 444-7270
Portland, OR (503) 636-4338
Washington, D.C. (301) 577-7830

NBU York 212-972-1500

Matrix Code: GW - Groundwater SL - Soil SD - Sediment SW - Surface water
OTHER - Please identify codes

Priority: ☑ Normal ☐ Rush Rush time period

Shipped via: ☑ FedEx/UPS ☐ Courier Other

Condition of Samples Upon Receipt:

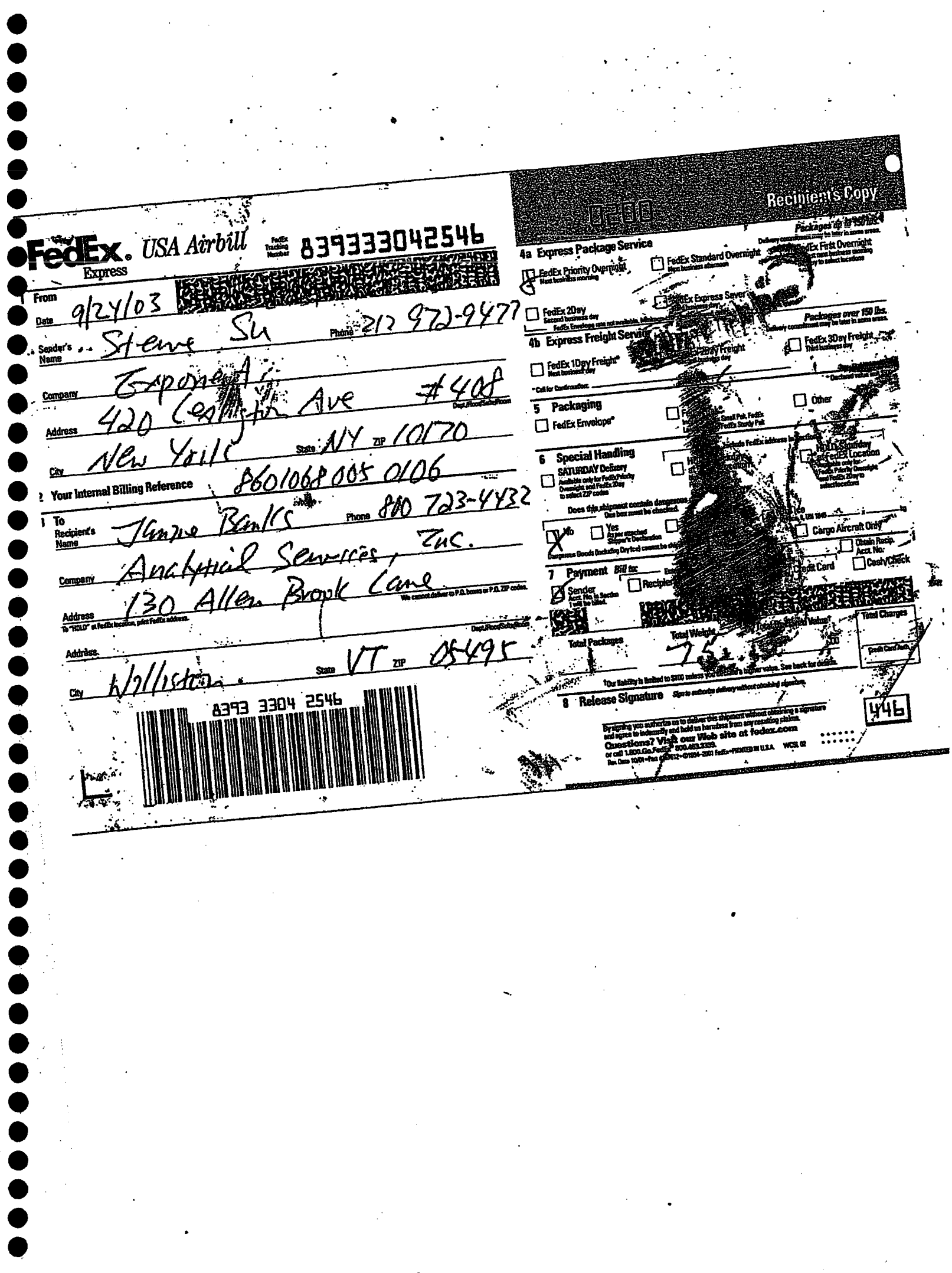
Custody Seal Intact: ☐ Yes ☐ No ☐ None

Relinquished by: (Signature) Date/Time: 9/24/03 3:00PM Received by: (Signature) Date/Time: 9/25/03 1115

Relinquished by: (Signature) Date/Time: Received by: (Signature) Date/Time:

Distribution: White and Yellow Copies - Accompany Shipment; Pink Copy - Project File

05815



FedEx. USA Airbill
Express

FedEx Tracking Number 839333042546

0290

Recipient's Copy

From

Date 9/24/03

Sender's Name Steve Su Phone 212 972-9477

Company Exponential

Address 420 Lexington Ave #408

Dept./Floor/Suite/Room

City New York State NY ZIP 10170

2 Your Internal Billing Reference 860106B005 0106

To

Recipient's Name James Banks Phone 800 723-4432

Company Analytical Services, Inc.

Address 130 Allen Brook Lane

We cannot deliver to P.O. boxes or P.O. ZIP codes.

To "HOLD" at FedEx location, print FedEx address.

Address

Dept./Floor/Suite/Room

City Williston State VT ZIP 05495

8393 3304 2546

4a Express Package Service

Packages up to 150 lbs.

☒ FedEx Priority Overnight — Next business morning

☐ FedEx Standard Overnight — Next business afternoon

☐ FedEx First Overnight — Earliest next business morning delivery to select locations

☐ FedEx Express Saver — Third business day

☐ FedEx 2Day — Second business day

FedEx Envelope rate not available. Minimum charges apply.

4b Express Freight Service

Packages over 150 lbs.

☐ FedEx 1Day Freight* — Next business day

☐ FedEx 2Day Freight — Second business day

☐ FedEx 3Day Freight — Third business day

*Call for Confirmation.

5 Packaging

☐ FedEx Envelope*

☐ FedEx Pak*

☐ FedEx Box

☐ FedEx Tube

☐ Other

*Declared value limit $500

6 Special Handling

SATURDAY Delivery

Available only for FedEx Priority Overnight and FedEx 2Day to select ZIP codes

☐ HOLD Weekday at FedEx Location

Not available for FedEx Priority Overnight or FedEx 2Day to select locations

☐ HOLD Saturday at FedEx Location

Available only for FedEx Priority Overnight and FedEx 2Day to select locations

Does this shipment contain dangerous goods?

One box must be checked.

☒ No

☐ Yes — As per attached Shipper's Declaration

Dangerous Goods (including Dry Ice) cannot be shipped in FedEx packaging.

☐ Cargo Aircraft Only

7 Payment Bill to:

☒ Sender — Acct. No. in Section 1 will be billed.

☐ Recipient

☐ Credit Card

☐ Cash/Check

Total Packages

Total Weight 75

Total Declared Value*

Total Charges

Credit Card Auth.

*Our liability is limited to $100 unless you declare a higher value. See back for details.

8 Release Signature Sign to authorize delivery without obtaining signature.

By signing you authorize us to deliver this shipment without obtaining a signature and agree to indemnify and hold us harmless from any resulting claims.

Questions? Visit our Web site at fedex.com or call 1.800.GoFedEx 800.463.3339.

Rev. Date 10/01 • Part #152612-©1994-2001 FedEx • PRINTED IN U.S.A. WCSL 02

446

Exponent

OFFICIAL SAMPLE SEAL

SAMPLE NO.	DATE
CSOL-SM-CF0303 CSOL-SM-CF0308	9/24/03
SIGNATURE	
PRINT NAME AND TITLE STAFF SCI, SSW. SCIENTIST	

Analytical Services Inc.
Method 1623 Bench Sheet

2003-0925-012/013

ASI Sample Identification #: 2003-0925-014 **Batch#:** 236-1

Client: EXPONENT **Lab Due Date:** 10/9/03

Client Sample ID: 63147

Sample Type (circle one): (field) IPR OPR MS method blank PE

Matrix (circle one): reagent water (raw surface water) finished water groundwater other: _____

Sample Collection: Date: 9/23/03 Time: 1215 **Received within holding time?** (YES) NO

Sample Turbidity, in NTU: **Temp °C upon receipt:** 5° (YES)

Sample Volume >10L? (YES) NO **Notes:**

Sample Spiking Date: 9/25/03 Analyst: JH Time: 1405

Spike suspension batch number: CS-ESWD-39

Sample volume spiked (L): 10.0

Estimated number spiked: cysts: 99±14 oocysts: 94±13

Filtration Date: 9/25/03 Analyst: JH Start time: 1607

Filter & Manufacturer: Gelman Envirochek (Gelman HV) Genera Filta Max Lot #: A1031430

Sample volume filtered, to nearest ¼ L: ~~10.0~~ JH 11:25/03 5.0 Rinsate volume, to nearest ¼ L*: 1.0

Did filter clog? (NO) ~~YES~~ JH 11:25/03 If Yes, 2nd volume filtered (L): 5.0

Elution / Centrifugation Date: 9/26/03 Analyst: SEC Start Time: 0913

Packed pellet volume (mL): ~~5.0~~ 2.5 2nd Packed pellet volume (mL): 0.6

Resuspended concentrate volume (mL): 250 2nd Resuspended concentrate volume (mL): 75

IMS / Staining – Volume Transferred to IMS

Sample volume, slide A:	5.0 mL (1.0 L)	Sample volume, slide B:	5.0 mL (1.0 L)
Sample volume, slide C:	5.0 mL (1.0 L)	Sample volume, slide D:	5.0 mL (1.0 L)
Sample applied to slide:	Date: 9/26/03	Analyst: JF/SEC/JH	Time: 1517
Staining completed:	Date: 9/26/03	Analyst: SEC	End Time: 1710

E: 5.0

COMMENTS:

-014 Spike with Color Seed

$\frac{2.5ml}{5.0L} = \frac{0.5}{X}$ X = 1.0 $\frac{2.5ml}{250ml} = \frac{0.5}{X}$ X = 50ml

-014-1 ~~5.0~~ ~~0.5~~ $\frac{0.6ml}{5.0L} = \frac{X}{CSF}$ 092603

Invoice Updated By:

*For IPR, OPR, MS AND MDL only. (spiked in accordance with USEPA Method 1623)

s:\Client\Smplmgmt\Workshts\1623

014-1 A 3.5ml (2.5L)
B 3.5ml (2.5L)

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-DAA
10-mL subsample ID (if packed pellet > 0.5 mL): A	Volume examined (in L) on this slide: 1.0
Analyst: C. Fregeau	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1	Oval	14.0X8.5		✓			✓			
2	↓	13.0X7.0			4			2		
3		13.0X7.5			4		✓			
4		13.0X7.5		✓			✓			
5		14.0X8.5			4			3		✓
6		13.0X9.5			3			2		
7		13.0X8.5		✓	4			3		
8		13.0X7.0		✓				2		✓
9	↓	14.0X9.5		✓			✓			
10	✓	14.0X7.0		✓				1		

Total FA number from this slide: 44	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): ~~1306~~ 0738 COF 100903
DAPI-: Total number (A): Ø	D.I.C.: Total number of empty cysts (D): Ø
DAPI+: Total number (B): 5	D.I.C.; Total number of cysts with amorphous structure (E): 4
DAPI+: Total number (C): 5	D.I.C.: Total number of cysts with one internal structure (F): 4
Total number DAPI + (C) that show structure by D.I.C. (F): 4	D.I.C.; Total number of cysts with >one internal structure (F): 2

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-0149
10-mL subsample ID (if packed pellet > 0.5 mL): A	Volume examined (in L) on this slide: 10
Analyst: C. Fregeau	Positive staining control acceptable ☒ YES ☐ NO Negative staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +	D.I.C.			
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	Round	5.0 x 5.0		✓			✓	
2								
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 1	Examination completion date: 100203
DAPI -: Total number (A): 0	Examination completion time (must be complete within 7 days of staining): 1306
DAPI +: Total number (B): 1	D.I.C. - Total number of empty oocysts (D): 0
DAPI +: Total number (C): 0	D.I.C. - Total number of oocysts with amorphous structure (E): 1
Total count DAPI + (C) that show structure by D.I.C. (F): 0	D.I.C. - Total number of oocysts with internal structure (F): 0

0728 CF 100907

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014B
10-mL subsample ID (if packed pellet > 0.5 mL): b	Volume examined (in L) on this slide: 1.0
Analyst: C. Fregeau	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 278	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 0802
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014B
10-mL subsample ID (if packed pellet > 0.5 mL): B	Volume examined (in L) on this slide: 1.0
Analyst: C. Fregeau	Positive staining control acceptable: ☑ YES ☐ NO Negative staining control acceptable: ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	Round	5.0x5.0		✓	4			3
2	↓	5.0x5.0		✓				1
3	↓	5.0x5.0			4			2
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 3	Examination completion date: 100203
DAPI -: Total number (A): Ø	Examination completion time (must be complete within 7 days of staining): 0802
DAPI +: Total number (B): 1	D.I.C. - Total number of empty oocysts (D): Ø
DAPI +: Total number (C): 2	D.I.C. - Total number of oocysts with amorphous structure (E): Ø
Total count DAPI + (C) that show structure by D.I.C. (F): 2	D.I.C. - Total number of oocysts with internal structure (F): 3

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014C
10-mL subsample ID (if packed pellet > 0.5 mL): C	Volume examined (in L) on this slide: 1.0
Analyst: C. Freyaer	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 113	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 0958
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0995-014C
10-mL subsample ID (if packed pellet > 0.5 mL): C	Volume examined (in L) on this slide: 1.0
Analyst: C-Freycon	Positive staining control acceptable ☑ YES ☐ NO Negative staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	Round	5.0x5.0			4			1
2	↓	4.5x4.5			4		✓	
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 2	Examination completion date: 100203
DAPI -: Total number (A): 0	Examination completion time (must be complete within 7 days of staining): 0958
DAPI +: Total number (B): 0	D.I.C. - Total number of empty oocysts (D): 0
DAPI +: Total number (C): 2	D.I.C. - Total number of oocysts with amorphous structure (E): 1
Total count DAPI + (C) that show structure by D.I.C. (F): 1	D.I.C. - Total number of oocysts with internal structure (F): 1

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 0003-0925-014D
10-mL subsample ID (if packed pellet > 0.5 mL): D	Volume examined (in L) on this slide: 1.0
Analyst: C. Fregeau	Pos. staining control acceptable ☒ YES ☐ NO Neg. staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 345	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1053
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-01FD
10-mL subsample ID (if packed pellet > 0.5 mL): D	Volume examined (in L) on this slide: 1.0
Analyst:	Positive staining control acceptable ☐ YES ☐ NO Negative staining control acceptable ☐ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	round	5.0x5.0		✓			✓	
2	↓	4.5X4.5			4		✓	
3		5.0 X 5.0			4			2
4		5.0 X 5.0			4		✓	
5	✓	5.0 X 5.0			4			1
6								
7								
8								
9								
10								

Total FA number from this slide: 5	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1053
DAPI -: Total number (A): 0	D.I.C. - Total number of empty oocysts (D): 0
DAPI +: Total number (B): 1	D.I.C. - Total number of oocysts with amorphous structure (E): 3
DAPI +: Total number (C): 4	D.I.C. - Total number of oocysts with internal structure (F): 2
Total count DAPI + (C) that show structure by D.I.C. (F): 2	

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014E
10-mL subsample ID (if packed pellet > 0.5 mL): E	Volume examined (in L) on this slide: 1.0
Analyst: C. Freyeem	Pos. staining control acceptable ☒ YES ☐ NO Neg. staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 119	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1108
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 030325-0925-014E
10-mL subsample ID (if packed pellet > 0.5 mL): E	Volume examined (in L) on this slide: 1.0
Analyst:	Positive staining control acceptable ☐ YES ☐ NO Negative staining control acceptable ☐ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: Ø	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1108
DAPI -: Total number (A):	D.I.C. - Total number of empty oocysts (D):
DAPI +: Total number (B):	D.I.C. - Total number of oocysts with amorphous structure (E):
DAPI +: Total number (C):	D.I.C. - Total number of oocysts with internal structure (F):
Total count DAPI + (C) that show structure by D.I.C. (F):	

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014-1A
10-mL subsample ID (if packed pellet > 0.5 mL): -1A	Volume examined (in L) on this slide: 2.5
Analyst: C. Freyleun	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 249	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1515
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

EK 9/26/03

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014-1A
10-mL subsample ID (if packed pellet > 0.5 mL): -1A	Volume examined (in L) on this slide: 0.5
Analyst: C. Fregeau	Positive staining control acceptable ☒ YES ☐ NO Negative staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 3	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1515
DAPI -: Total number (A):	D.I.C. - Total number of empty oocysts (D):
DAPI +: Total number (B):	D.I.C. - Total number of oocysts with amorphous structure (E):
DAPI +: Total number (C):	D.I.C. - Total number of oocysts with internal structure (F):
Total count DAPI + (C) that show structure by D.I.C. (F):	

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-014-1B
10-mL subsample ID (if packed pellet > 0.5 mL): -1B	Volume examined (in L) on this slide: 99
Analyst: C. Freyaer	Pos. staining control acceptable ☒ YES ☐ NO Neg. staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 722	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1535
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 0003-0925-014-1B
10-mL subsample ID (if packed pellet > 0.5 mL): -1B	Volume examined (in L) on this slide: 2.5
Analyst: C-Freyaun	Positive staining control acceptable ☑ YES ☐ NO Negative staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 2	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1535
DAPI -: Total number (A):	D.I.C. - Total number of empty oocysts (D):
DAPI +: Total number (B):	D.I.C. - Total number of oocysts with amorphous structure (E):
DAPI +: Total number (C):	D.I.C. - Total number of oocysts with internal structure (F):
Total count DAPI + (C) that show structure by D.I.C. (F):	

See back for details.
CJF

2003-0925-014
Exponent
63147

	Total G	Total C	C.S G	C.S C	
14 A	44	~~0~~ OF 1	1	1	0738
B	278	3	3	3	0802
C	113	2	~~0~~	2	0958
D	345	5	~~0~~	~~0~~ OF 5	1053
E	119	~~0~~	2	~~0~~	1108
14-1 A	249	3	2	3	1515
14-1 B	722	2	3	2	1535
Total	1870	16	11	16	

Ing.
G - 1859
C - ~~0~~

Seeded	Recovered	%
98G	11	11.2
99C	16	16.2

Analytical Services Inc.
Method 1623 Bench Sheet

2003-0925-012/013

ASI Sample Identification #: 2003-0925-015 **Batch#:** 236-1

Client: EXPONENT, INC **Lab Due Date:** 10/9/03

Client Sample ID: 63161

Sample Type (circle one): (field) IPR OPR MS method blank PE

Matrix (circle one): reagent water (raw surface water) finished water groundwater other: _____

Sample Collection: Date: 9/23/03 Time: 1230 **Received within holding time?** (YES) NO

Sample Turbidity, in NTU: **Temp °C upon receipt:** 5°

Sample Volume >10L? (YES) NO **Notes:**

Sample Spiking

Date: 09263 CJF 09263 Analyst: CJF CJF Time: 0846

Spike suspension batch number: CS-CB103-39

Sample volume spiked (L): 10.0

Estimated number spiked*: Cysts: 994 ± 12 Oocysts: 994 ± 13

Filtration

Date: CJF 9/26/03 Analyst: CJF CJF Start time: 0847

Filter & Manufacturer: Gelman Envirochek (Gelman HV) Genera Filta Max **Lot #:** A1031 4303

Sample volume filtered, to nearest ¼ L: 10.0 **Rinsate volume, to nearest ¼ L*:** 1.0

Did filter clog? (NO) YES **If Yes, 2nd volume filtered (L):**

Elution / Centrifugation

Date: 9/26/03 **Analyst:** SEC **Start Time:** 0913

Packed pellet volume (mL): 1.0 **2nd Packed pellet volume (mL):**

Resuspended concentrate volume (mL): 10.0 **2nd Resuspended concentrate volume (mL):**

IMS / Staining – Volume Transferred to IMS

Sample volume, slide A: 5.0 mL (5.0 L) **Sample volume, slide B:** 5.0 mL (5.0 L)

Sample volume, slide C: mL (L) **Sample volume, slide D:** mL (L)

Sample applied to slide: Date: 9/26/03 Analyst: CJF/SEC/SA Time: 1517

Staining completed: Date: 9/26/03 Analyst: SEC **End Time:** 1710

COMMENTS:

Spike with Color Seed

Invoice Updated By:

*For IPR, OPR, MS AND MDL only. (spiked in accordance with USEPA Method 1623)

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Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0929-015A
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 25.0L
Analyst: C. Freyeu	Pos. staining control acceptable ☒ YES ☐ NO Neg. staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1	Oval	13.0x8.5		✓			✓			
2	↓	14.0x7.5			4		✓			
3		13.0x7.0			4			2		
4		13.0x8.5		✓				1		
5		12.0x8.5		✓				3		✓
6		13.0x9.5		✓				1		
7		13.0x8.5			3			1		
8		14.0x8.5		✓			✓			
9		14.0x9.5		✓				1		
10	↓	14.0x7.0		✓			✓			

Total FA number from this slide: 460	Examination completion date: 100203
	Examination completion time (must be complete within 7 days of staining): 1549
DAPI-: Total number (A): 0	D.I.C.: Total number of empty cysts (D): 0
DAPI+: Total number (B): 7	D.I.C.: Total number of cysts with amorphous structure (E): 4
DAPI+: Total number (C): 3	D.I.C.: Total number of cysts with one internal structure (F): 5
Total number DAPI + (C) that show structure by D.I.C. (F): 2	D.I.C.: Total number of cysts with >one internal structure (F): 1

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-092S-015A
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 5.0
Analyst: C. Freyeau	Positive staining control acceptable ☒ YES ☐ NO Negative staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	round	5.0x5.0		✓			✓	
2	↓	5.0x5.0		✓				2
3	↓	5.0x5.0		✓			✓	
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: 3	Examination completion date: 100203
DAPI -: Total number (A): 0	Examination completion time (must be complete within 7 days of staining): 1549
DAPI +: Total number (B): 3	D.I.C. - Total number of empty oocysts (D): 0
DAPI +: Total number (C): 0	D.I.C. - Total number of oocysts with amorphous structure (E): 2
Total count DAPI + (C) that show structure by D.I.C. (F): 0	D.I.C. - Total number of oocysts with internal structure (F): 1

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): QM03-0925-015B
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 5.0
Analyst: C. Freyeu	Pos. staining control acceptable ☒ YES ☐ NO Neg. staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: 342	Examination completion date: 00203
	Examination completion time (must be complete within 7 days of staining): 1609
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D):
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 0003-0925-015B
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 5.0
Analyst: C-Freyeer	Positive staining control acceptable ☒ YES ☐ NO Negative staining control acceptable ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	Round	5.0x5.0		✓				1
2	↓	5.0x5.0			4		✓	
3		5.0x5.0		✓			✓	
4		5.0x5.0			3			3
5	↓	5.0x5.0			4			1
6								
7								
8								
9								
10								

Total FA number from this slide:	5	Examination completion date:	100203
		Examination completion time (must be complete within 7 days of staining):	1609
DAPI -: Total number (A):	0	D.I.C. - Total number of empty oocysts (D):	0
DAPI +: Total number (B):	2	D.I.C. - Total number of oocysts with amorphous structure (E):	2
DAPI +: Total number (C):	3	D.I.C. - Total number of oocysts with internal structure (F):	3
Total count DAPI + (C) that show structure by D.I.C. (F):	2		

see back for details
CJF

2003-0925-015

Exponent

63161

		Total G	Total C	C.S. G	C.S. C	Indig. G	Indig. C
1549	15A	460	3	~~0~~	3	460	~~0~~
1609	15B	342	5	4	5	338	~~0~~
		802	8	4	8	798	~~0~~

Seeded	Recovered	%
98 G	4	4.1 G
99 C	8	8.1 C

Ing.

G → 798

C → ~~0~~

Analytical Services Inc.
Method 1623 Bench Sheet

ASI Sample Identification #: 2003-0925-012				Batch#: 236-1	
Client: ASE				Lab Due Date: 10/3/03	
Client Sample ID: ASI 236-1 OPR					
Sample Type (circle one): field	IPR	(OPR)	MS	method blank	PE
Matrix (circle one): (reagent water)	raw surface water	finished water	groundwater	other: _____	
Sample Collection: Date: 9/25/03	Time:		Received within holding time? (YES)	NO	
Sample Turbidity, in NTU :			Temp °C upon receipt:		
Sample Volume >10L? YES	NO	Notes:			
Sample Spiking* Date: 9/25/03	Analyst: CJF		Time: 1930		
Spike suspension batch number: ES-Cry 102-132-					
Sample volume spiked (L): 10.0 L					
Estimated number spiked: cysts: 1001.2	Oocysts: 955.07				
Filtration Date: 9/25/03	Analyst: JH		Start time: 1535		
Filter & Manufacturer: Gelman Envirochek	(Gelman HV)	Genera Filta Max	Lot #: A1031430 3		
Sample volume filtered, to nearest ¼ L: 10.0			Rinsate volume, to nearest ¼ L*: 1.0		
Did filter clog? (NO) YES			If Yes, 2nd volume filtered (L):		
Elution / Centrifugation Date: 9/26/03	Analyst: SEC		Start Time: 0815		
Packed pellet volume (mL): Trace			2nd Packed pellet volume (mL):		
Resuspended concentrate volume (mL): 5.0			2nd Resuspended concentrate volume (mL):		
IMS / Staining – Volume Transferred to IMS					
Sample volume, slide A: 5.0 mL (10.0 L)			Sample volume, slide B: mL (L)		
Sample volume, slide C: mL (L)			Sample volume, slide D: mL (L)		
Sample applied to slide: Date: 9/26/03	Analyst: CJF/SEC/JH		Time: 1500		
Staining completed: Date: 9/26/03	Analyst: SEC		End Time: 1710		

COMMENTS:

Invoice Updated By: ✓

*For IPR, OPR, MS AND MDL only. (spiked in accordance with USEPA Method 1623)

s:\Client\Smplmgmt\Workshts\1623

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-012
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 10.0
Analyst: S. Cabral	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1	Oval	13.0 X 8.5	✓				✓			
2	Oval	14.5X 9.5	✓				✓			
3	Oval	14.0X 7.5	✓				✓			
4	Oval	13.0X7.5	✓				✓			
5	Oval	13.0X 8.5	✓				✓			
6	Oval	13.0X 9.5	✓				✓			
7	Oval	13.0X8.5	✓				✓			
8	Oval	14.0X 9.5	✓				✓			
9	Oval	14.5X8.5		✓			✓			
10	Round	13.0X 10.5		✓			✓			

Total FA number from this slide: 72	Examination completion date: 9/29/03
DAPI-: Total number (A): 8	Examination completion time (must be complete within 7 days of staining): 1445
DAPI+: Total number (B): 8	D.I.C.: Total number of empty cysts (D): 0
DAPI+: Total number (C): 0	D.I.C.: Total number of cysts with amorphous structure (E): 10
Total number DAPI + (C) that show structure by D.I.C. (F): 0	D.I.C.: Total number of cysts with one internal structure (F): 0
	D.I.C.: Total number of cysts with >one internal structure (F): 0

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-0925-012
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 10.0
Analyst: S. Caldwal	Positive staining control acceptable ~~□~~ YES □ NO Negative staining control acceptable ~~□~~ YES □ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +		D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1	Round	4.0 x 4.5	✓				✓	
2	Round	4.5 x 4.5	✓				✓	
3	Round	5.0 x 4.5		✓			✓	
4	Round	5.0 x 5.0		✓			✓	
5	Round	5.0 x 5.0		✓			✓	
6	Round	5.0 x 5.0	✓				✓	
7	Round	5.0 x 4.5		✓			✓	
8	Round	5.0 x 4.5	✓				✓	
9	Round	4.5 x 4.5	✓				✓	
10	Round	5.0 x 5.0	✓				✓	

Total FA number from this slide: 45	Examination completion date: 9/29/03
DAPI -: Total number (A): 6	Examination completion time (must be complete within 7 days of staining): 1445
DAPI +: Total number (B): 4	D.I.C. - Total number of empty oocysts (D): 0
DAPI +: Total number (C): 0	D.I.C. - Total number of oocysts with amorphous structure (E): 10
Total count DAPI + (C) that show structure by D.I.C. (F): 0	D.I.C. - Total number of oocysts with internal structure (F): 0

Analytical Services Inc.
Method 1623 Bench Sheet

ASI Sample Identification #: 2003-0925-013					Batch#: 236-1	
Client: ASR					Lab Due Date: 10/3/03	
Client Sample ID: ASI 236-1 BLK						
Sample Type (circle one):	field	IPR	OPR	MS	(method blank)	PE
Matrix (circle one): (reagent water)	raw surface water	finished water	groundwater	other:		
Sample Collection: Date: 9/25/03	Time:			Received within holding time?	(YES)	NO
Sample Turbidity, in NTU:				Temp °C upon receipt:		
Sample Volume >10L?	YES	NO	Notes:			
Sample Spiking:	Date:		Analyst:		Time:	
Spike suspension batch number:						
Sample volume spiked (L):						
Estimated number spiked:	Cysts:		Oocysts:			
Filtration	Date: 9/25/03		Analyst: JH		Start time: 1544	
Filter & Manufacturer:	Gelman Envirochek	(Gelman HV)	Genera Filta Max		Lot #: A1031 4303	
Sample volume filtered, to nearest ¼ L: 10.0			Rinsate volume, to nearest ¼ L*: N/A			
Did filter clog? (NO)	YES		If Yes, 2nd volume filtered (L):			
Elution / Centrifugation	Date: 9/26/03		Analyst: SEC		Start Time: 0913	
Packed pellet volume (mL): Trace			2nd Packed pellet volume (mL):			
Resuspended concentrate volume (mL): 5.0			2nd Resuspended concentrate volume (mL):			
IMS / Staining – Volume Transferred to IMS						
Sample volume, slide A: 5.0 mL (10.0 L)			Sample volume, slide B:		mL (	L)
Sample volume, slide C:	mL (	L)	Sample volume, slide D:		mL (	L)
Sample applied to slide:	Date: 9/26/03		Analyst: GJF/SEC/JH		Time: 1500	
Staining completed:	Date: 9/26/03		Analyst: SEC		End Time: 1710	

COMMENTS:

Invoice Updated By: ✓

*For IPR, OPR, MS AND MDL only. (spiked in accordance with USEPA Method 1623)

Laboratory name:	Laboratory ID (if applicable):

Method 1623 *Giardia* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 2003-025-013
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 10.0
Analyst: S. Cabral	Pos. staining control acceptable ☑ YES ☐ NO Neg. staining control acceptable ☑ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (μm)	DAPI -	DAPI +		D.I.C.				
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty cysts (D)	Cysts with amorphous structure (E)	Cysts with internal structure (F)		
								Number of nuclei	Median body	Axonemes
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Total FA number from this slide: Ø	Examination completion date: 16/1/03
	Examination completion time (must be complete within 7 days of staining): 0930
DAPI-: Total number (A):	D.I.C.: Total number of empty cysts (D): Ø
DAPI+: Total number (B):	D.I.C.: Total number of cysts with amorphous structure (E):
DAPI+: Total number (C):	D.I.C.: Total number of cysts with one internal structure (F):
Total number DAPI + (C) that show structure by D.I.C. (F):	D.I.C.: Total number of cysts with >one internal structure (F):

Laboratory name:	Laboratory ID:

Method 1622/1623 *Cryptosporidium* Report Form

Client sample number:	Internal laboratory sample ID (if applicable): 0603-0925-013
10-mL subsample ID (if packed pellet > 0.5 mL):	Volume examined (in L) on this slide: 10.0
Analyst: S. Cabral	Positive staining control acceptable: ☒ YES ☐ NO Negative staining control acceptable: ☒ YES ☐ NO

Object located by FA No.	Shape (oval or round)	Size L x W (µm)	DAPI -	DAPI +	D.I.C.		
			Light blue internal staining, no distinct nuclei, green rim (A)	Intense blue internal staining (B)	Number of nuclei stained sky blue (C)	Empty oocysts (D)	Oocysts with amorphous structure (E)	Oocysts with internal structure (F) Number of sporozoites
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Total FA number from this slide: Ø	Examination completion date: 10/1/03
DAPI -: Total number (A):	Examination completion time (must be complete within 7 days of staining): 0930
DAPI +: Total number (B):	D.I.C. - Total number of empty oocysts (D): Ø
DAPI +: Total number (C):	D.I.C. - Total number of oocysts with amorphous structure (E):
Total count DAPI + (C) that show structure by D.I.C. (F):	D.I.C. - Total number of oocysts with internal structure (F):

EMSL Analytical, Inc.
107 Haddon Avenue, Westmont, NJ. 08108 (856) 858-4800

Client: Exponent

New York
Attn. Frank Brockerhoff
Project: Passaic River

EMSL Reference: 370304632
Date Received: 9/23/03
Date Analyzed: 9/23/03 @ 7:00
Date Reported: 10/7/03

Membrane Filtration Testing
Modified Membrane Filtration Methods SM 9222B, 9222D, 9222G, 9230C

Sample	Location	*Fecal Streptococcus* CFU/100 ml	Total Coliform CFU/100 ml	E. coli Positive/Negative	Fecal Coliform CFU/100 ml
CS001-SAY-0923-03		>30,000	>30,000	Positive	>30,000
CS002-SAY-0923-03		>30,000	>30,000	Positive	>30,000

Approved EMSL Signatory
Hilisa Esteban PhD, Laboratory Manager

NJ Certification # 04006

EMSL Analytical, Inc.
107 Haddon Avenue, Westmont, NJ. 08108 (856) 858-4800

Client: Exponent

New York
Attn. Frank Brockerhoff
Project: Passaic River

EMSL Reference: 370304632
Date Received: 9/23/03
Date Analyzed: 10/3/03
Date Reported: 10/7/03

Bacterial Identification
For the Analysis of Water Samples By Agar Culture

Sample	Location	Size of Sample Area	Bacterial Identification	Concentration (CFU/ml)
CS001-SAY-0923-03			Citrobacter freundii	3000
			Aeromonas ichthiosmia	5000
			Pantoea dispersa	4000
			Kluyvera ascorbata	3000
			Microbacterium lacticum	3000
			Actinomycetes	8000
			Gram Variable Rods	2000
CS002-SAY-0923-03			Gram Negative Rods resembling Enterobacter species	4000
			Gram Variable Rods resembling Myroides odoratus	3000
			Rathayibacter/Rothia species	10000
			Enterobacter cloacae	2000
			Gram Negative Rods resembling Pantoea species	2000
			Actinomycetes	10000
			Gram Positive Rods	3000
			Gram Variable Rods	1000
			Acinetobacter species	3000

CFU = Colony Forming Unit

Concentration is reported in CFU's/Swab unless otherwise noted

AIHA EMLAP Lab ID # 100194

Approved EMSL Signatory
Hilisa Esteban PhD, Laboratory Manager

CHAIN OF CUSTODY RECORD/SAMPLE ANALYSIS REQUEST FORM

3703046.32 X37 EXPONY

Page 1 of 1

Project: (Name and Number) PASSAIC RIVER NON-RC/FS 860/068.005.0106

Exponent Contact: STEVE SU Office: NY

Ship to: EMSL ANALYTICAL, INC.
107 Haddon Avenue
Westmont, NJ 08108

Lab Contact/Phone: Hilda Esteban 800-220-3675

Samplers: FRANK BROCKOWOFF

Exponent
Environmental Group
Bellevue, WA (425) 643-9803
Boston, MA (781) 466-6681
Boulder, CO (303) 444-7270
Portland, OR (503) 636-4338
Washington, D.C. (301) 577-7830
NEW YORK (212) 972-1500

Sample No.	Tag No.	Date	Time	Matrix	Analyses Requested: Standard Plate Count with 2D mdlt	Standard Method 9222 mdl3	Extra Container	Archive	Remarks
CS001-SAY-0923-03	63147	9/22/03	12:45P	SW	✓	✓			
CS002-SAY-0923-03	63153	9/23/03	7:30	SW	✓	✓			

SAMPLES ACCEPTED FOR ANALYSIS BY EMSL ANALYTICAL INC.

03 SEP 23 PM 1:17

PU 9/23/03

Matrix Code: GW - Groundwater SL - Soil SD - Sediment SW - Surface water
OTHER - Please identify codes

Priority: ☑ Normal ☐ Rush Rush time period

Shipped via: ☐ FedEx/UPS ☑ Courier Other

Condition of Samples Upon Receipt:

Custody Seal Intact: ☐ Yes ☐ No ☐ None

Relinquished by: (Signature) Date/Time: 9/22/03 4:40PM Received by: (Signature) Date/Time: 9/23/03 4:39

Relinquished by: (Signature) Date/Time: Received by: (Signature) Date/Time:

Distribution: White and Yellow Copies - Accompany Shipment; Pink Copy - Project File

05811

Benchsheet for Total Coliform, Fecal Coliform, E.coli

Company: Client Exponent Test: M025 Sewage Screen #Samples: 2
Project #: Order: 370304632 Project: #3601068.005.0106/PASSAIC RIVER
Reference #: Disposition: *Discard after 11/22/03*

Sample Number	Matrix	Amount Sampled	Colilert (Presence/Absence)		Preliminary Membrane Filtration		Verification Membrane Filtration						Final Results (per 100ml)
			Total Coliform[1]	E.coli[2]	Total Coliform # colonies	Fecal Coliform # colonies	LTB[3]		BGB[3]		EC Mug[2]	EC Medium[3]	
			24Hrs	24hrs	24 hrs	24 hours	24 hrs	48 hr	24 hrs	48 hr	24 hrs	24 hrs	
1		0.5ml			>300	70	+		+		+	+	⊕ TC, FC, Ecoli, B
		1ml			>300	>300							
2		0.5ml			>300	>300	+		+		+	+	⊕ TC, FC Ecoli, B
		1ml			>300	>300							

Legend:
1 Yellow color change (+), No color change (-) 3 No Gas(-), Gas (+)
2 Fluorescents (FL), non-fluorescent (NFL)

Record Lot Numbers Here:

SetUp-Analyst Signature: SD Date/Time: 9/23 200

24hr read-Analyst Signature: SD Date/Time: 9/24 800

48hr read-Analyst Signature: SD Date/Time: 9/25 800

9/26 3pm

9/27 600

9/24 sw

Page____ of ____.
J: worksheets/TCFCEcoli

Benchsheet for Fecal Streptococcus

Company: Exponent
Project #: #9601068.005.0106/PASSAIC RIVER
Reference: 9/23/03 TAT 144+ Hours
M025 Sewage Screen Water

Order ID: 370304632
No Samples: 2
Due: 10/03 3:47 PM
Fax:

Sample Number	Matrix	Amount Sampled	Preliminary	Verification						
			Fecal Strep # colonies 48 hours	Gram Stain	Catalase +/-	BHI Broth[1] 45°C 48 hrs	BHI Broth[1] +6.5%NaCl 35°C 48hrs	Bile Esculin Agar +/-[2] 48hrs	Commercial Test Kit or System	Final Results
1			7300		(-)	+	+	+		
2			7300		(-)	+	+	+		

SM 9230C Membrane Filtration (or Modification of SM for matrix other than water

Legend:
1 Growth (G), No Growth (NG) 2 Black(+), No color (-)

Record Lot Numbers Here:

SetUp-Analyst Signature: Date/Time:

24hr read-Analyst Signature: Date/Time: 9/26 3:30

48hr read-Analyst Signature: Date/Time:

EMSL Analytical Inc., 107 Haddon Avenue, Westmont, NJ 08052

Date Received 9/23/03
Client Name Exponent
Project Name #8601068.005.0106/Passaic River
EMSL Reference # 370304632

BACTERIAL ANALYSIS

(Label here) (Melissa Krows Contact; Steve Su)

Full speciation

2 samples

C. freundii

BIOLOG

GVR — MIDI x

48 hr reading						Final reading						
Date	Analyst	Sample #	Dilution	#	Identification	Date	Analyst	Sample #	Dilution	Changes?	#	Identification
9/26	g	1	1000*	3	off wh muc (1)	Myroides odoratus MIDI			— GVR	*	3	pale mustard (6)
		MIDI — GNR	(TSBA)*	5	cream muc (2)	Rahnella? sp.			BIOL	GPR	1	yell wt (7)
5)		MIDI — GNR *		4	gold-yell (3)	sp.		MIDI	— GVR	*	10	tiny wh (8)
		Pantoea agglomerans	(NOS)	1	yell wt (4)	Acinetobacter sp.		MIDI	— GVR	*	3	white (9)
— = predominant			GVR	1	off wh wt (5)			BIOL			10	Actinos
			GVR	1	irreg muc bge (6)	G-S + Iso all — work up 5 predom + for						
		XMIDI — GNR *		3	grey (7)	9/29	g	Re-iso 1 — (1)(2)(3)(7)(8) significant				
		MIDI — GNR *		3	tiny yell (8)			Re-iso 2 — (1)(2)(4)(6)(8)(9)(11)				
		BIOL?		8	Actinos	10/1	jc	Iso to MIDI plates → Biolog				
Kluyvera/Pantoea			(NOS)	2	white (9)			plates (prob. Enterob.) when ox⊖				
MIDI — GNR		2	1000*	4	grey (1)	10/2	jc	BIOL — 1,3,7,8 & 2-1,2,4				
BIOL Pantoea?		Kluyvera ascorb.	(TSBA)	2	vr grey (2)	GNR — MIDI — all GNent; 2-8,9: GNNent.						
		*Pick for ...		2	lt must (3)	GPR						
BIOL? Pantoea		Enterobacter/Pantoea		2	golden yell (4)	GNR — MIDI						
			GVR	1	egg-yolk yell (5)							

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Comments:

Analyst jc

Date Received 9/23/03
Client Name Exponent
Project Name #860106.8.005.0106/Passaic River
EMSL Reference # 370304632

BACTERIAL ANALYSIS

(Label here)

FINAL ~~48 hr~~ reading

Date	Analyst	Sample #	Dilution	#	Identification
10/3	gf	1	1000	3	Citrobacter Freundii
				5	Aeromonas ichthiosmia
				4	Pantoea dispersa
				3	Kluyvera ascorbata
				3	Microbacterium lacticum
				8	Actinomycetes
				2	gram variable rods.

Final reading

Date	Analyst	Sample #	Dilution	Changes?	#	Identification
		2	1000	—	4	GNR resembling Enterobacter sp
					3	gram variable rods resembling Myroides odoratus
					10	Rathayibacter/ Rothia species
					2	Enterobacter cloacae
					2	GNR resembling Pantoea sp.
					10	Actinomycetes
					3	gram ⊖ rods
					1	gram var. rods
					3	Acinetobacter species

cfu/ml = count x dilution

Comments:

Analyst gf

PAGE 05/35
EMSL ANALYTICAL
8568589551
13:06
02/23/2004

E03A027.55A [1474] 4632-1, 1

Volume: DATA File: E03A027.55A Seq Counter: 5 ID Number: 1474
Type: Samp Bottle: 4 Method: TSBA50
Created: 10/2/03 7:46:05 PM
Sample ID: 4632-1, 1

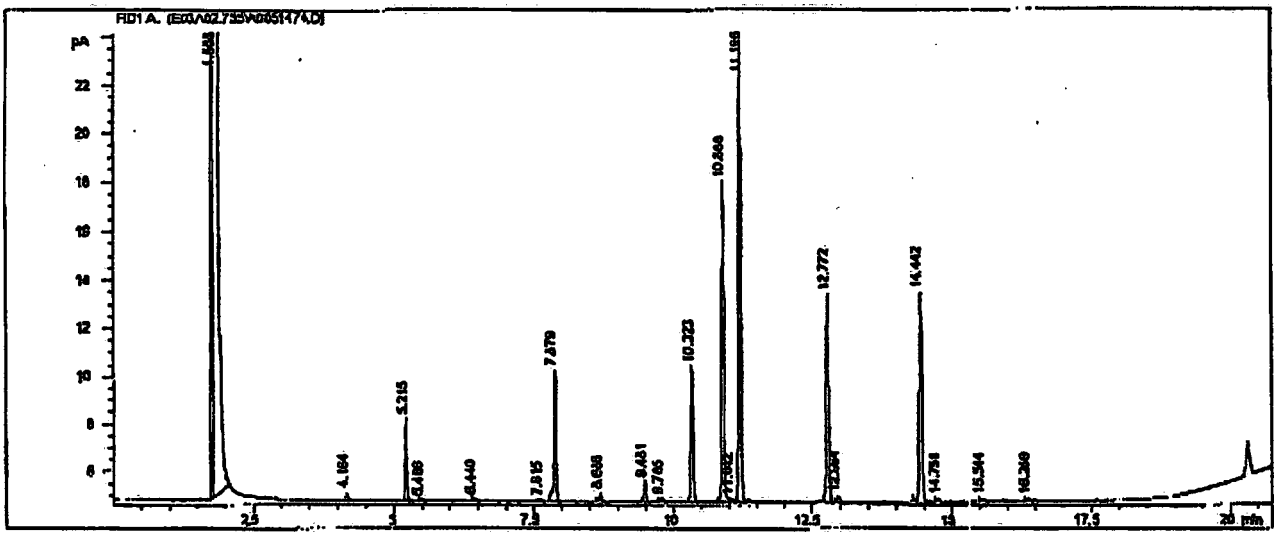
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.768	195833	0.017	---	6.939		---	< min rt	
1.805	3.532E+8	0.033	---	7.005	SOLVENT PEAK	---	< min rt	
4.164	1621	0.034	1.125	10.921	Sum In Feature 2	0.51	ECL deviates 0.007	12:0 ALDE ?
5.215	13746	0.031	1.073	12.000	12:0	4.17	ECL deviates 0.000	Reference -0.004
5.486	269	0.032	---	12.222		---		
6.440	592	0.039	1.033	13.000	13:0	0.17	ECL deviates 0.000	Reference -0.004
7.615	548	0.038	---	13.816		---		
7.879	30552	0.042	1.000	14.000	14:0	8.62	ECL deviates 0.000	Reference -0.004
8.688	2515	0.050	0.985	14.505	unknown 14.502	0.70	ECL deviates 0.003	
9.481	4621	0.042	0.971	15.000	15:0	---	ECL deviates 0.000	
9.785	1001	0.041	---	15.178		---		
10.323	32152	0.045	0.959	15.492	Sum In Feature 2	8.71	ECL deviates 0.004	14:0 3OH/16:1 ISO I
10.888	76374	0.044	0.951	15.821	Sum In Feature 3	20.52	ECL deviates -0.001	16:1 w7c/15 iso 2OH
11.042	839	0.042	0.949	15.911	16:1 w5c	0.23	ECL deviates 0.002	
11.196	106138	0.044	0.947	16.000	16:0	28.40	ECL deviates 0.000	Reference -0.003
12.772	50985	0.046	0.930	16.891	17:0 CYCLO	13.39	ECL deviates 0.003	Reference 0.000
12.964	1369	0.046	0.928	16.999	17:0	0.36	ECL deviates -0.001	Reference -0.004
14.442	53551	0.048	0.914	17.825	18:1 w7c	13.82	ECL deviates 0.002	
14.751	950	0.041	0.911	17.997	18:0	0.24	ECL deviates -0.003	Reference -0.003
15.544	1069	0.050	---	18.444		---		
16.359	639	0.039	0.898	18.903	19:0 CYCLO w8c	0.16	ECL deviates 0.001	Reference 0.000
---	33773	---	---	---	Summed Feature 2	9.22	12:0 ALDE ?	unknown 10.928
---	---	---	---	---		---	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
---	76374	---	---	---	Summed Feature 3	20.52	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.003 Reference ECL Shift: 0.003 Number Reference Peaks: 8
Total Response: 374911 Total Named: 372024
Percent Named: 99.23% Total Amount: 358590

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.880	Salmonella-typhimurium-GC subgroup B
	0.745	Kluyvera-cryocrescens-GC subgroup B
	0.725	Pantoea-agglomerans-GC subgroup B (Enterobacter)
	0.550	Citrobacter-freundii
	0.544	Raoultella-terrigena (Klebsiella)

E03A027.55A [1474] 4632-1, 1



E03A027.55A [1475] 4632-1, 2 Page 1

Volume: DATA File: E03A027.55A Seq Counter: 6 ID Number: 1475
Type: Samp Bottle: 5 Method: TSBA50
Created: 10/2/03 8:11:05 PM
Sample ID: 4632-1, 2

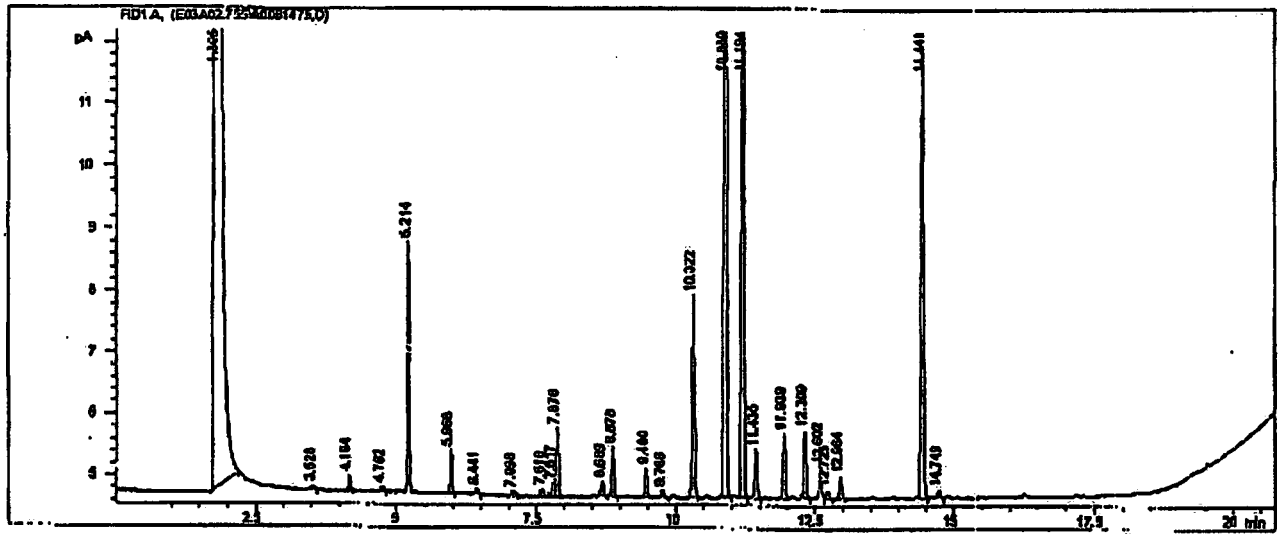
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.805	3.562E+8	0.029	—	7.001	SOLVENT PEAK	—	< min rt	
3.528	263	0.031	—	10.081		—		
4.164	1084	0.034	1.124	10.922	Sum In Feature 2	0.43	ECL deviates -0.006	unknown 10.928
4.762	298	0.031	1.094	11.544	unknown 11.543	0.11	ECL deviates 0.001	
5.214	16478	0.032	1.073	12.000	12:0	6.19	ECL deviates 0.000	Reference -0.004
5.968	2945	0.033	1.048	12.615	13:0 ISO	1.08	ECL deviates 0.001	Reference -0.002
6.441	368	0.033	1.033	13.000	13:0	0.13	ECL deviates 0.000	Reference -0.003
7.098	388	0.034	1.017	13.457	12:0 3OH	0.14	ECL deviates 0.003	
7.616	649	0.040	—	13.817		—		
7.817	1553	0.046	1.001	13.956	unknown 13.957	0.54	ECL deviates -0.001	
7.878	5675	0.040	1.000	13.998	14:0	1.98	ECL deviates -0.002	Reference -0.005
8.689	1311	0.044	0.985	14.506	unknown 14.502	0.45	ECL deviates 0.004	
8.878	3996	0.041	0.981	14.624	15:0 ISO	1.37	ECL deviates 0.001	Reference -0.003
9.480	2501	0.042	0.971	15.000	15:0	—	ECL deviates 0.000	
9.786	631	0.047	—	15.178		—		
10.322	18707	0.044	0.959	15.491	Sum In Feature 2	6.27	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.889	114456	0.045	0.951	15.822	Sum In Feature 3	38.08	ECL deviates 0.000	16:1 w7c/15 iso 2OH
11.194	64989	0.045	0.947	16.000	16:0	21.54	ECL deviates 0.000	Reference -0.004
11.435	4475	0.044	0.945	16.136	15:0 ISO 3OH	1.48	ECL deviates 0.002	
11.939	6096	0.047	0.939	16.421	ISO 17:1 w9c	2.00	ECL deviates 0.005	
12.309	6416	0.045	0.935	16.630	17:0 ISO	2.10	ECL deviates 0.000	Reference -0.004
12.602	2887	0.046	0.931	16.796	17:1 w8c	0.94	ECL deviates 0.004	
12.723	788	0.042	0.930	16.865	17:1 w6c	0.26	ECL deviates 0.005	
12.964	2041	0.046	0.928	17.001	17:0	0.66	ECL deviates 0.001	Reference -0.004
14.441	43865	0.048	0.914	17.827	18:1 w7c	14.02	ECL deviates 0.004	
14.749	702	0.043	0.911	17.999	18:0	0.22	ECL deviates -0.001	Reference -0.006
—	19790	—	—	—	Summed Feature 2	6.70	12:0 ALDE ?	unknown 10.928
—	—	—	—	—		—	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
—	114456	—	—	—	Summed Feature 3	38.08	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.003 Reference ECL Shift: 0.004 Number Reference Peaks: 9
Total Response: 301062 Total Named: 299519
Percent Named: 99.49% Total Amount: 288351

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.814	Aeromonas-ichthiosmia A/hydrophila
	0.735	Aeromonas-caviae
	0.722	Aeromonas-hydrophila/ichthiosmia A/sobria
	0.690	Aeromonas-trota/enteropelogenes
	0.606	Aeromonas-veronii-GC subgroup B (biogroup sobria)
	0.578	Aeromonas-jandaei
	0.510	Aeromonas-veronii-GC subgroup A (biogroup veronii)

E03A027.55A [1475] 4632-1, 2



E03A027.55A [1476] 4632-1, 3 Page 1

Volume: DATA File: E03A027.55A Seq Counter: 7 ID Number: 1476
Type: Samp Bottle: 6 Method: TSBA50
Created: 10/2/03 8:36:02 PM
Sample ID: 4632-1, 3

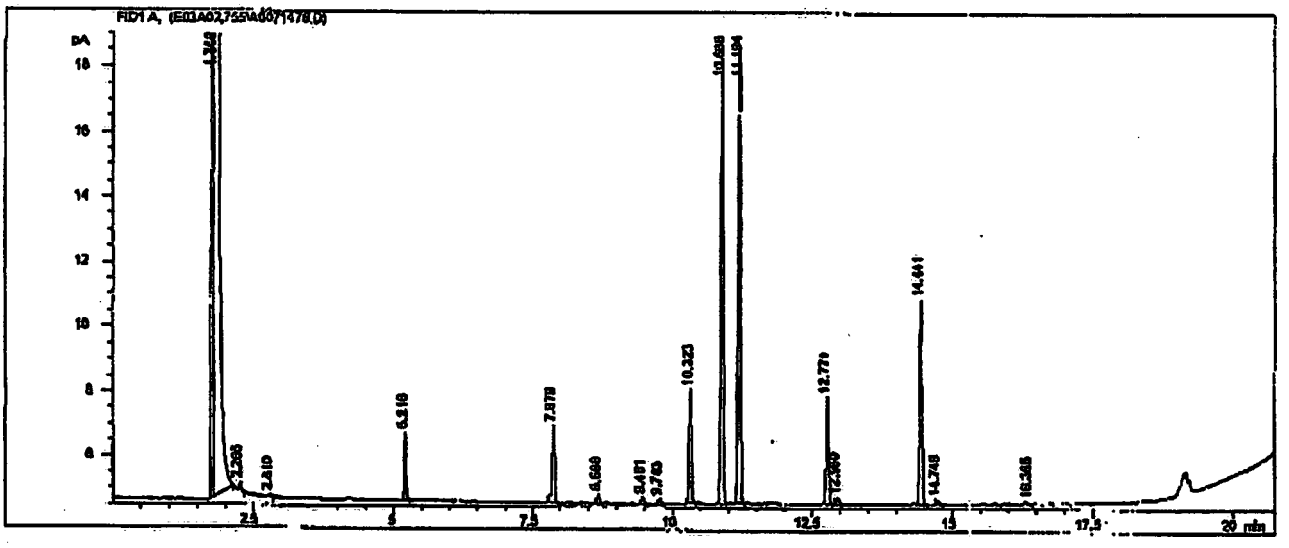
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.769	345819	0.018	---	6.941		---	< min rt	
1.806	3.56E+8	0.033	---	7.007	SOLVENT PEAK	---	< min rt	
2.265	951	0.028	---	7.835		---	< min rt	
2.810	191	0.020	---	8.818		---	< min rt	
5.216	8459	0.032	1.073	11.999	12:0	3.70	ECL deviates -0.001	Reference -0.003
7.878	13908	0.042	1.000	14.001	14:0	5.66	ECL deviates 0.001	Reference -0.004
8.688	1573	0.045	0.985	14.507	unknown 14.502	0.63	ECL deviates 0.005	
9.481	1083	0.043	0.971	15.002	15:0	---	ECL deviates 0.002	
9.783	778	0.039	---	15.178		---		
10.323	19799	0.044	0.959	15.493	Sum In Feature 2	7.73	ECL deviates 0.005	14:0 3OH/16:1 ISO I
10.888	76753	0.045	0.951	15.823	Sum In Feature 3	29.72	ECL deviates 0.001	16:1 w7c/15 iso 2OH
11.194	78059	0.045	0.947	16.001	16:0	30.10	ECL deviates 0.001	Reference -0.004
12.771	19987	0.046	0.930	16.891	17:0 CYCLO	7.56	ECL deviates 0.003	Reference -0.001
12.960	1257	0.044	0.928	16.998	17:0	0.47	ECL deviates -0.002	Reference -0.006
14.441	37608	0.047	0.914	17.824	18:1 w7c	13.98	ECL deviates 0.001	
14.749	767	0.043	0.911	17.996	18:0	0.28	ECL deviates -0.004	Reference -0.006
16.365	445	0.044	0.898	18.905	19:0 CYCLO w8c	0.16	ECL deviates 0.003	Reference 0.003
---	19799	---	---	---	Summed Feature 2	7.73	12:0 ALDE ?	unknown 10.928
---	---	---	---	---		---	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
---	76753	---	---	---	Summed Feature 3	29.72	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.003 Reference ECL Shift: 0.004 Number Reference Peaks: 7
Total Response: 259393 Total Named: 258615
Percent Named: 99.70% Total Amount: 246759

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.914	Pantoea-agglomerans-GC subgroup A (Entb. agglom., Er. herbic.)
	0.830	Serratia-grimesii
	0.828	Enterobacter-intermedius
	0.748	Serratia-odorifera
	0.746	Brenneria-rubrifaciens
	0.655	Yersinia-frederiksenii
	0.601	Raoultella-terrigena (Klebsiella)
	0.595	Salmonella-typhimurium-GC subgroup B
	0.594	Pantoea-agglomerans-GC subgroup E (Enterobacter)
	0.589	Achromobacter-xylosoxidans-denitrificans (Alcaligenes)

E03A027.55A [1476] 4632-1, 3



E03A027.55A [1477] 4632-1, 7

Volume: DATA File: E03A027.55A Seq Counter: 8 ID Number: 1477
Type: Samp Bottle: 7 Method: TSBA50
Created: 10/2/03 9:01:04 PM
Sample ID: 4632-1, 7

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.768	161878	0.017	---	6.942		---	< min rt	
1.805	3.56E+8	0.033	---	7.007	SOLVENT PEAK	---	< min rt	
2.611	492	0.025	---	8.459		---	< min rt	
4.162	1018	0.030	1.125	10.920	Sum In Feature 2	0.34	ECL deviates 0.006	12:0 AI.DE ?
5.214	12012	0.032	1.073	12.000	12:0	3.78	ECL deviates 0.000	Reference -0.004
6.698	3944	0.037	1.027	13.180	12:0 2OH	1.19	ECL deviates 0.003	
7.878	37088	0.042	1.000	13.999	14:0	10.88	ECL deviates -0.001	Reference -0.004
8.688	1904	0.041	0.985	14.505	unknown 14.502	0.55	ECL deviates 0.003	
9.480	879	0.041	0.971	14.999	15:0	---	ECL deviates -0.001	
9.783	1462	0.047	---	15.176		---		
10.323	38010	0.043	0.959	15.491	Sum In Feature 2	10.70	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.887	47683	0.045	0.951	15.819	Sum In Feature 3	13.31	ECL deviates -0.003	16:1 w7c/15 iso 2OH
11.042	688	0.039	0.949	15.910	16:1 w5c	0.19	ECL deviates 0.001	
11.195	103025	0.043	0.947	15.999	16:0	28.64	ECL deviates -0.001	Reference -0.003
12.772	59971	0.046	0.930	16.890	17:0 CYCLO	16.36	ECL deviates 0.002	Reference 0.000
14.311	611	0.034	---	17.751		---		
14.441	49430	0.048	0.914	17.823	18:1 w7c	13.25	ECL deviates 0.000	
15.545	1019	0.050	---	18.444		---		
16.358	3048	0.045	0.898	18.902	19:0 CYCLO w8c	0.80	ECL deviates 0.000	Reference -0.001
---	39028	---	---	---	Summed Feature 2	11.03	12:0 AI.DE ?	unknown 10.928
---	---	---	---	---		---	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
---	47683	---	---	---	Summed Feature 3	13.31	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.002
Total Response: 360914
Percent Named: 99.14%

Reference ECL Shift: 0.003
Total Named: 357822
Total Amount: 341631

Number Reference Peaks: 5

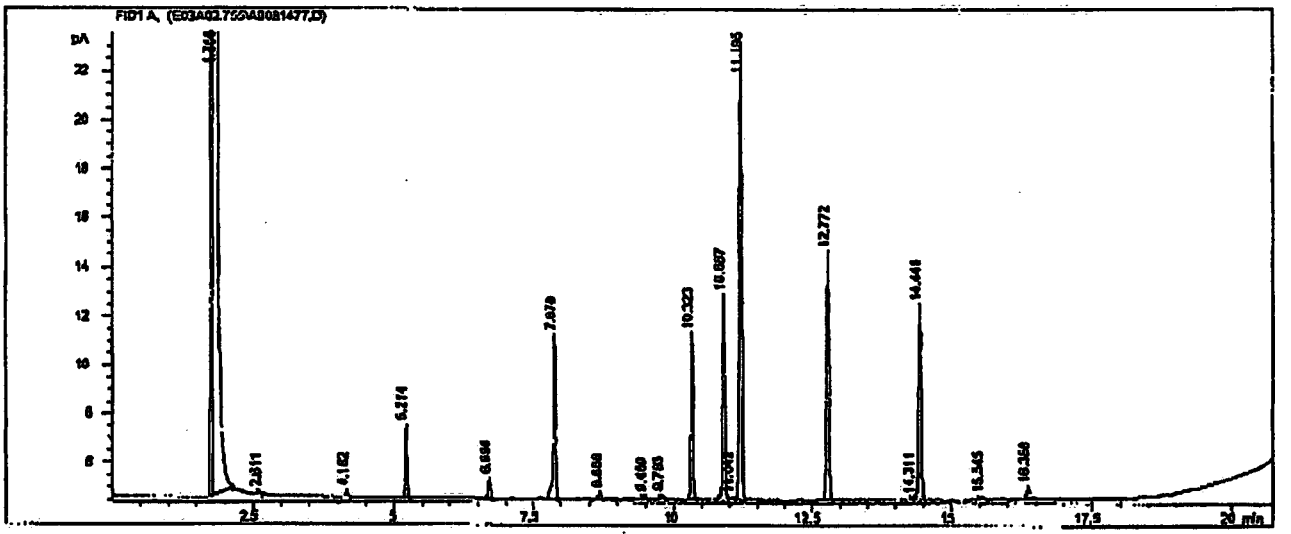
Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.743	Salmonella-typhimurium-GC subgroup B
	0.693	Kluyvera-ascorbata-GC subgroup B
	0.665	Klebsiella-pneumoniae-ozaenae-GC subgroup B
	0.518	Klebsiella-pneumoniae-pneumoniae-GC subgroup A

Sherlock Version 4.5 (0209B)

Printed on 02-Oct-2003

E03A027.55A [1477] 4632-1, 7



E03A027.55A [1478] 4632-1, 8

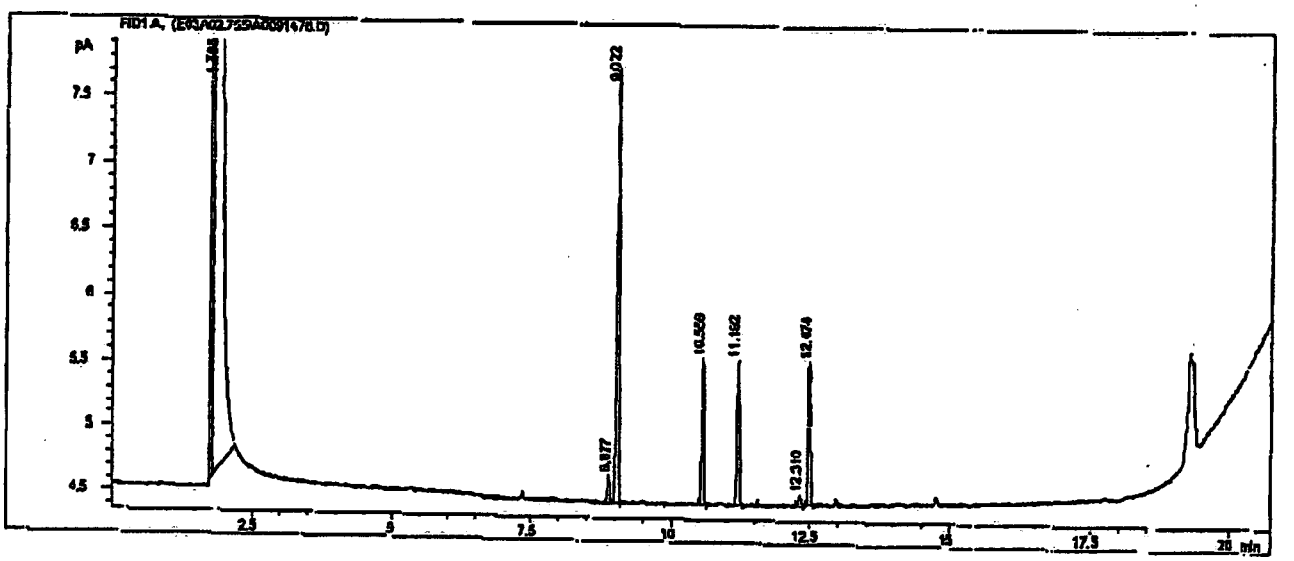
Volume: DATA File: E03A027.55A Seq Counter: 9 ID Number: 1478
Type: Samp Bottle: 8 Method: TSBA50
Created: 10/2/03 9:25:57 PM
Sample ID: 4632-1, 8

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.768	179436	0.017	---	6.936		---	< min rt	
1.805	3.596E+8	0.033	---	7.002	SOLVENT PEAK	---	< min rt	
8.877	1021	0.035	0.981	14.623	15:0 ISO	2.79	ECL deviates 0.000	Reference -0.003
9.022	17269	0.042	0.979	14.713	15:0 ANTEISO	47.01	ECL deviates 0.000	Reference -0.003
10.556	6112	0.043	0.956	15.628	16:0 ISO	16.25	ECL deviates 0.001	Reference -0.003
11.192	6215	0.044	0.947	15.999	16:0	16.38	ECL deviates -0.001	Reference -0.005
12.310	422	0.040	0.935	16.631	17:0 ISO	1.10	ECL deviates 0.001	Reference -0.003
12.474	6349	0.044	0.933	16.723	17:0 ANTEISO	16.47	ECL deviates 0.000	Reference -0.003

ECL Deviation: 0.001 Reference ECL Shift: 0.003 Number Reference Peaks: 6
Total Response: 37389 Total Named: 37389
Percent Named: 100.00% Total Amount: 35954
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.790	Microbacterium-lacticum-GC subgroup A
	0.640	Clavibacter-michiganensis-nebraskensis/C. tessellarius



4632-1 (8)

E03A036.06A [1492] conc # 8

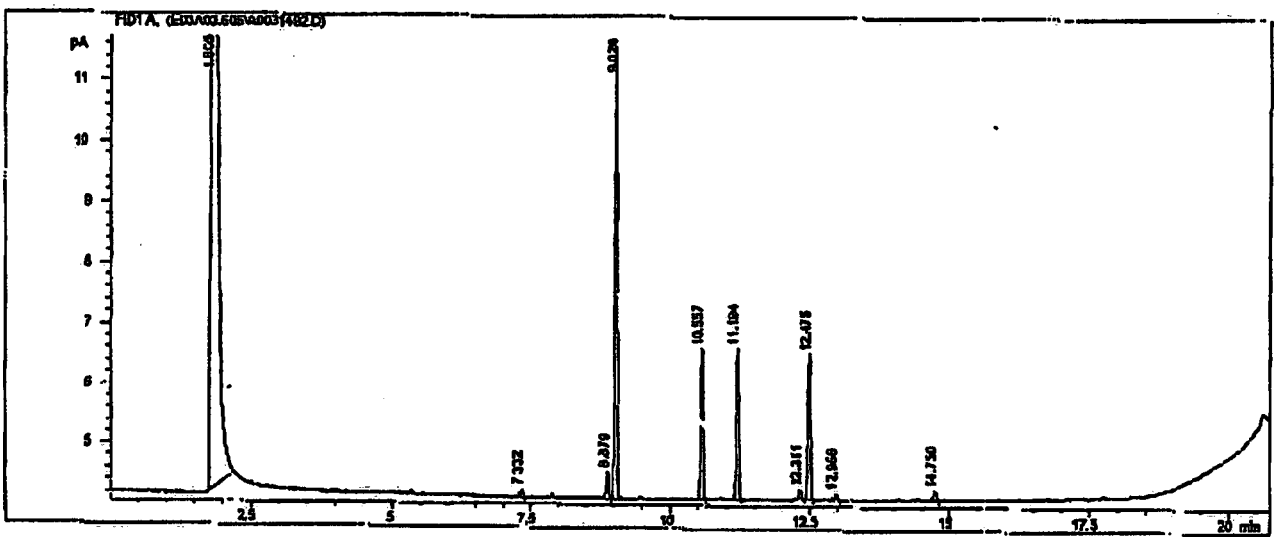
Volume: DATA File: E03A036.06A Seq Counter: 3 ID Number: 1492
Type: Samp Bottle: 2 Method: TSBA50
Created: 10/3/03 3:20:34 PM
Sample ID: conc # 8

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.805	3.71E+8	0.027	----	7.000	SOLVENT PEAK	----	< min rt	
7.332	652	0.036	1.006	13.619	14:0 ISO	0.80	ECL deviates 0.000	Reference -0.002
8.879	2380	0.042	0.979	14.623	15:0 ISO	2.86	ECL deviates 0.000	Reference -0.001
9.024	38060	0.040	0.977	14.713	15:0 ANTEISO	45.60	ECL deviates 0.000	Reference -0.001
10.557	13508	0.043	0.958	15.627	16:0 ISO	15.86	ECL deviates 0.000	Reference -0.001
11.194	13739	0.043	0.951	15.998	16:0	16.02	ECL deviates -0.002	Reference -0.003
12.311	1004	0.047	0.940	16.631	17:0 ISO	1.16	ECL deviates 0.001	Reference -0.001
12.475	13900	0.047	0.938	16.723	17:0 ANTEISO	16.00	ECL deviates 0.000	Reference -0.001
12.966	591	0.040	0.934	17.001	17:0	0.68	ECL deviates 0.001	Reference 0.000
14.750	908	0.043	0.921	17.999	18:0	1.03	ECL deviates -0.001	Reference -0.003

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 9
Total Response: 84742 Total Named: 84742
Percent Named: 100.00% Total Amount: 81545

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.895	Microbacterium-lacticum-GC subgroup A
	0.548	Clavibacter-michiganensis-nebraskensis/C. tessellarius



E03A027.55A [1479] 4632-2, 1

Volume: DATA File: E03A027.55A Seq Counter: 10 ID Number: 1479
Type: Samp Bottle: 9 Method: TSBA50
Created: 10/2/03 9:50:53 PM
Sample ID: 4632-2, 1

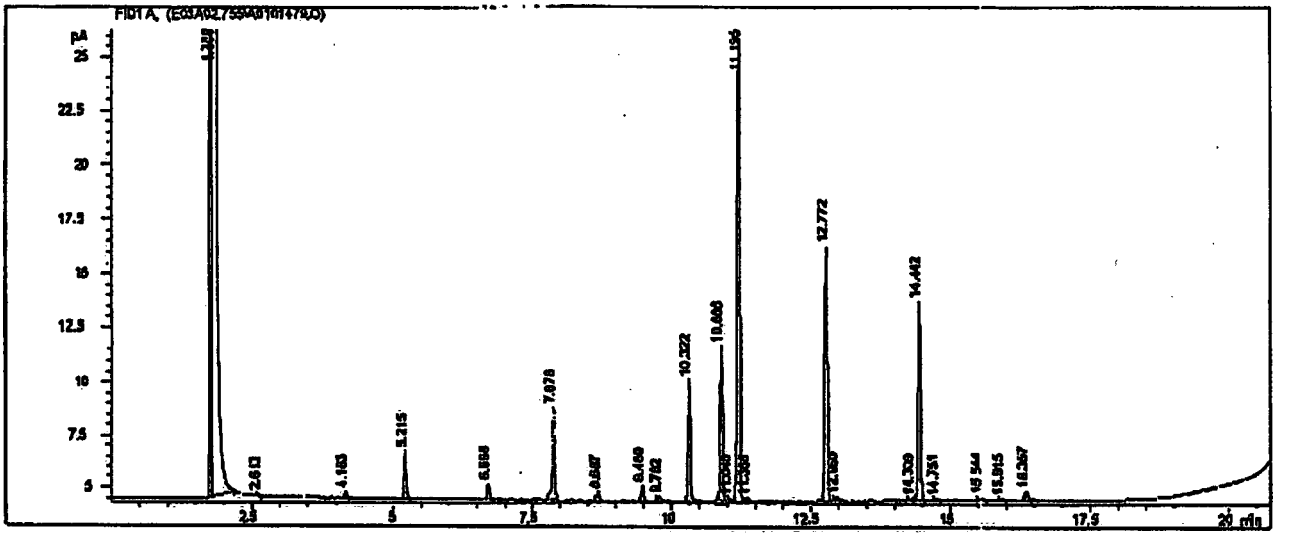
RT	Response	Ar/It	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.769	116159	0.017	—	6.940		—	< min rt	
1.806	3.607E+8	0.033	—	7.006	SOLVENT PEAK	—	< min rt	
2.613	462	0.026	—	8.461		—	< min rt	
4.163	1249	0.033	1.125	10.920	Sum In Feature 2	0.41	ECL deviates 0.006	12:0 ALDE ?
5.215	9168	0.033	1.073	12.000	12:0	2.85	ECL deviates 0.000	Reference -0.004
6.698	3748	0.037	1.027	13.179	12:0 2OH	1.11	ECL deviates 0.002	
7.878	25042	0.043	1.000	14.000	14:0	7.25	ECL deviates 0.000	Reference -0.004
8.687	2224	0.044	0.985	14.505	unknown 14.502	0.63	ECL deviates 0.003	
9.480	3879	0.043	0.971	15.000	15:0	—	ECL deviates 0.000	
9.782	1270	0.044	—	15.176		—		
10.322	31948	0.043	0.959	15.491	Sum In Feature 2	8.87	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.886	40582	0.043	0.951	15.820	Sum In Feature 3	11.18	ECL deviates -0.002	16:1 w7c/15 iso 2OH
11.040	657	0.041	0.949	15.910	16:1 w5c	0.18	ECL deviates 0.001	
11.195	119486	0.043	0.947	16.000	16:0	32.79	ECL deviates 0.000	Reference -0.003
11.356	1064	0.059	—	16.092		—		
12.772	69836	0.046	0.930	16.891	17:0 CYCLO	18.80	ECL deviates 0.003	Reference 0.000
12.960	1188	0.046	0.928	16.998	17:0	0.32	ECL deviates -0.002	Reference -0.006
14.309	874	0.042	—	17.751		—		
14.442	55443	0.047	0.914	17.825	18:1 w7c	14.67	ECL deviates 0.002	
14.751	695	0.043	0.911	17.998	18:0	0.18	ECL deviates -0.002	Reference -0.005
15.544	1208	0.049	—	18.445		—		
15.915	486	0.034	—	18.654		—		
16.357	2873	0.048	0.898	18.903	19:0 CYCLO w8c	0.75	ECL deviates 0.001	Reference -0.002
—	33198	—	—	—	Summed Feature 2	9.28	12:0 ALDE ?	unknown 10.928
—	—	—	—	—		—	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
—	40582	—	—	—	Summed Feature 3	11.18	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.002 Reference ECL Shift: 0.004 Number Reference Peaks: 7
Total Response: 369040 Total Named: 364138
Percent Named: 98.67% Total Amount: 349062

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.722	Kluyvera-ascorbata-GC subgroup B
	0.686	Pantoea-agglomerans-GC subgroup B (Enterobacter)
	0.629	Cedecea-davisae
	0.621	Klebsiella-pneumoniae-ozaenae-GC subgroup B
	0.610	Salmonella-typhi-GC subgroup A (confirm with other tests)
	0.586	Escherichia-coli-GC subgroup E (DNA homology with Shigella)
	0.563	Pantoea-agglomerans-GC subgroup C (Enterobacter)
	0.554	Salmonella-typhimurium-GC subgroup B
	0.517	Raoultella-terrigena (Klebsiella)
	0.508	Enterobacter-gergoviae

E03A027.55A [1479] 4632-2, 1



E03A027.55A [1480] 4632-2, 2 Page 1

Volume: DATA File: E03A027.55A Seq Counter: 11 ID Number: 1480
Type: Samp Bottle: 10 Method: TSBA50
Created: 10/2/03 10:15:55 PM
Sample ID: 4632-2, 2

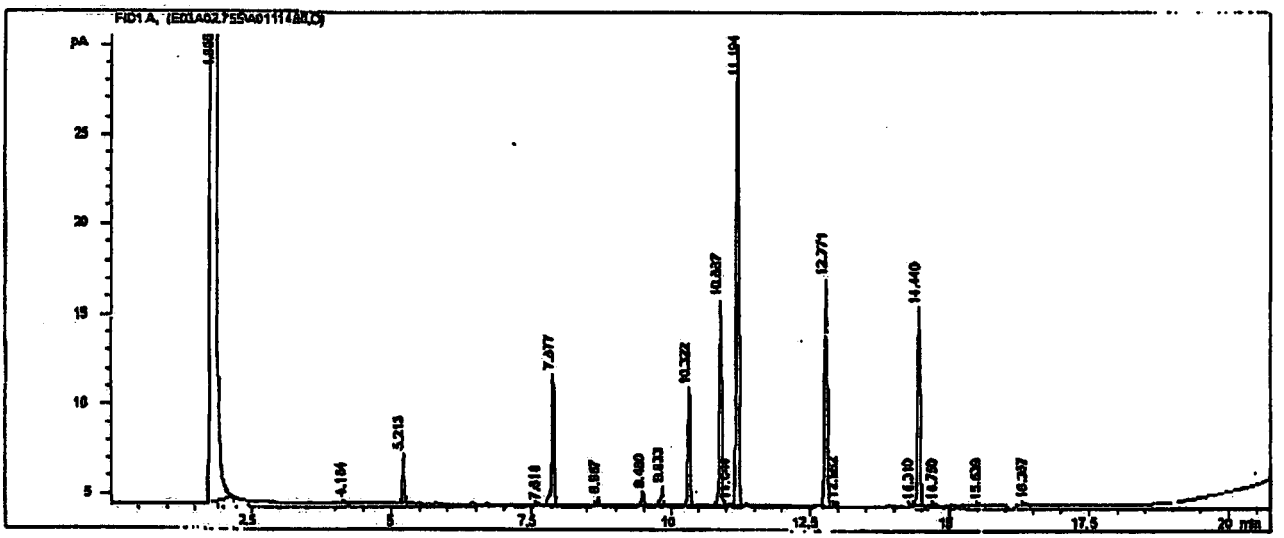
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.805	3.587E+8	0.029	—	7.006	SOLVENT PEAK	—	< min rt	
4.164	861	0.035	1.124	10.922	Sum In Feature 2	0.23	ECL deviates -0.006	unknown 10.928
5.213	11217	0.032	1.073	12.000	12:0	2.82	ECL deviates 0.000	Reference -0.005
7.618	395	0.032	—	13.820		—		
7.877	39981	0.041	1.000	14.000	14:0	9.34	ECL deviates 0.000	Reference -0.005
8.687	2539	0.047	0.985	14.505	unknown 14.502	0.58	ECL deviates 0.003	
9.480	4211	0.043	0.971	15.000	15:0	—	ECL deviates 0.000	
9.833	7436	0.050	0.966	15.207	14:0 2OH	1.68	ECL deviates 0.004	
10.322	36826	0.045	0.959	15.491	Sum In Feature 2	8.26	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.887	65274	0.044	0.951	15.821	Sum In Feature 3	14.52	ECL deviates -0.001	16:1 w7c/15 iso 2OH
11.044	701	0.039	0.949	15.912	16:1 w5c	0.16	ECL deviates 0.003	
11.194	141139	0.043	0.947	16.000	16:0	31.27	ECL deviates 0.000	Reference -0.004
12.771	74114	0.046	0.930	16.891	17:0 CYCLO	16.11	ECL deviates 0.003	Reference 0.000
12.962	1306	0.042	0.928	16.998	17:0	0.28	ECL deviates -0.002	Reference -0.005
14.310	741	0.041	—	17.752		—		
14.440	67251	0.047	0.914	17.824	18:1 w7c	14.37	ECL deviates 0.001	
14.750	622	0.038	0.911	17.998	18:0	0.13	ECL deviates -0.002	Reference -0.003
15.539	1118	0.056	—	18.442		—		
16.357	1227	0.050	0.898	18.903	19:0 CYCLO w8c	0.26	ECL deviates 0.001	Reference -0.002
—	37687	—	—	—	Summed Feature 2	8.48	12:0 ALDE ?	unknown 10.928
—	—	—	—	—		—	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
—	65274	—	—	—	Summed Feature 3	14.52	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.003 Reference ECL Shift: 0.004 Number Reference Peaks: 7
Total Response: 452749 Total Named: 450496
Percent Named: 99.50% Total Amount: 431786

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.809	Kluyvera-ascorbata-GC subgroup B
	0.663	Klebsiella-pneumoniae-ozaenae-GC subgroup B
	0.632	Salmonella-typhimurium-GC subgroup B
	0.624	Enterobacter-asburiae
	0.604	Klebsiella-pneumoniae-pneumoniae-GC subgroup A
	0.603	Proteus-vulgaris
	0.596	Pantoea-agglomerans-GC subgroup B (Enterobacter)
	0.580	Serratia-rubidaea
	0.579	Salmonella-bongori
	0.551	Cedecea-davisae

E03A027.55A [1480] 4632-2, 2



E03A027.55A [1481] 4632-2, 4 Page 1

Volume: DATA File: E03A027.55A Seq Counter: 12 ID Number: 1481
Type: Samp Bottle: 11 Method: TSBA50
Created: 10/2/03 10:40:54 PM
Sample ID: 4632-2, 4

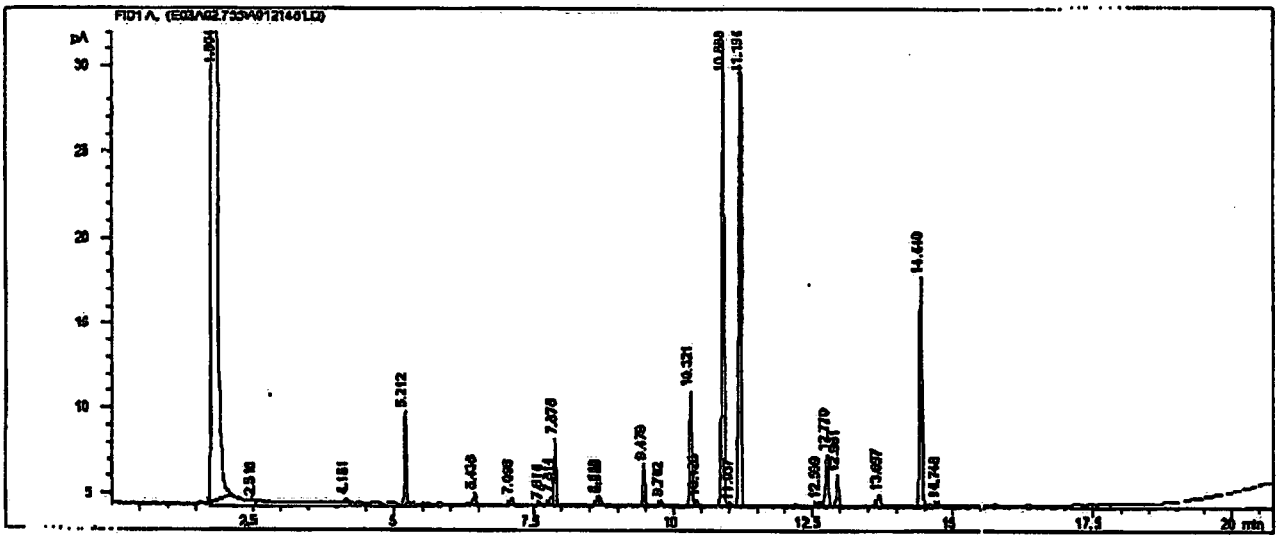
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.804	3.536E+8	0.029	—	7.005	SOLVENT PEAK	—	< min rt	
2.518	220	0.022	—	8.293		—	< min rt	
4.161	1058	0.035	1.124	10.923	Sum In Feature 2	0.25	ECL deviates -0.005	unknown 10.928
5.212	21637	0.033	1.073	12.000	12:0	4.80	ECL deviates 0.000	Reference -0.007
6.438	2831	0.036	1.033	13.000	13:0	0.61	ECL deviates 0.000	Reference -0.005
7.096	1804	0.042	1.017	13.458	12:0 3OH	0.38	ECL deviates 0.004	
7.614	487	0.035	—	13.817		—		
7.814	3467	0.045	1.001	13.956	unknown 13.957	0.72	ECL deviates -0.001	
7.876	19483	0.039	1.000	13.999	14:0	4.03	ECL deviates -0.001	Reference -0.005
8.638	2231	0.041	0.985	14.475	Sum In Feature 1	0.45	ECL deviates -0.003	15:1 ISO I/13:0 3OH
8.686	2505	0.041	0.985	14.505	unknown 14.502	0.51	ECL deviates 0.003	
9.479	12983	0.042	0.971	15.000	15:0	—	ECL deviates 0.000	
9.782	1620	0.041	—	15.177		—		
10.321	37694	0.045	0.959	15.491	Sum In Feature 2	7.48	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.426	1714	0.043	0.958	15.552	16:0 N alcohol	0.34	ECL deviates 0.002	
10.888	151948	0.044	0.951	15.821	Sum In Feature 3	29.90	ECL deviates -0.001	16:1 w7c/15 iso 2OH
11.037	520	0.036	0.949	15.908	16:1 w5c	0.10	ECL deviates -0.001	
11.194	145870	0.044	0.947	16.000	16:0	28.58	ECL deviates 0.000	Reference -0.004
12.599	1177	0.044	0.931	16.794	17:1 w8c	0.23	ECL deviates 0.002	
12.770	18931	0.049	0.930	16.891	17:0 CYCLO	3.64	ECL deviates 0.003	Reference -0.001
12.961	10271	0.045	0.928	16.999	17:0	1.97	ECL deviates -0.001	Reference -0.005
13.697	3946	0.049	0.920	17.411	17:0 10 methyl	0.75	ECL deviates 0.002	
14.440	79575	0.047	0.914	17.827	18:1 w7c	15.04	ECL deviates 0.004	
14.748	1219	0.041	0.911	17.999	18:0	0.23	ECL deviates -0.001	Reference -0.006
—	2231	—	—	—	Summed Feature 1	0.45	15:1 ISO H/13:0 3OH	13:0 3OH/15:1 I/H
—	—	—	—	—		—	15:1 ISO I/13:0 3OH	
—	38752	—	—	—	Summed Feature 2	7.72	12:0 ALDE ?	unknown 10.928
—	—	—	—	—		—	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
—	151948	—	—	—	Summed Feature 3	29.90	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1 w7c

ECL Deviation: 0.002 Reference ECL Shift: 0.005 Number Reference Peaks: 7
Total Response: 509990 Total Named: 507883
Percent Named: 99.59% Total Amount: 496124

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.713	Enterobacter-intermedius
	0.662	Pantoea-agglomerans-GC subgroup A (Entb. agglom., Er. herbic.)
	0.638	Pantoea-ananatis/Erwinia uredovora (E.ananatis)
	0.603	Serratia-odorifera
	0.544	Serratia-grimesii
	0.543	Yersinia-frederiksenii
	0.529	Brenneria-rubrifaciens
	0.476	Vibrio-hollisae
	0.475	Achromobacter-xylosoxidans-denitrificans (Alcaligenes)
	0.471	Erwinia-chrysanthemi-biotype II

E03A027.55A [1481] 4632-2, 4



E03A027.55A [1482] 4632-2, 6 Page 1

Volume: DATA File: E03A027.55A Seq Counter: 13 ID Number: 1482
Type: Samp Bottle: 12 Method: TSBA50
Created: 10/2/03 11:05:45 PM
Sample ID: 4632-2, 6

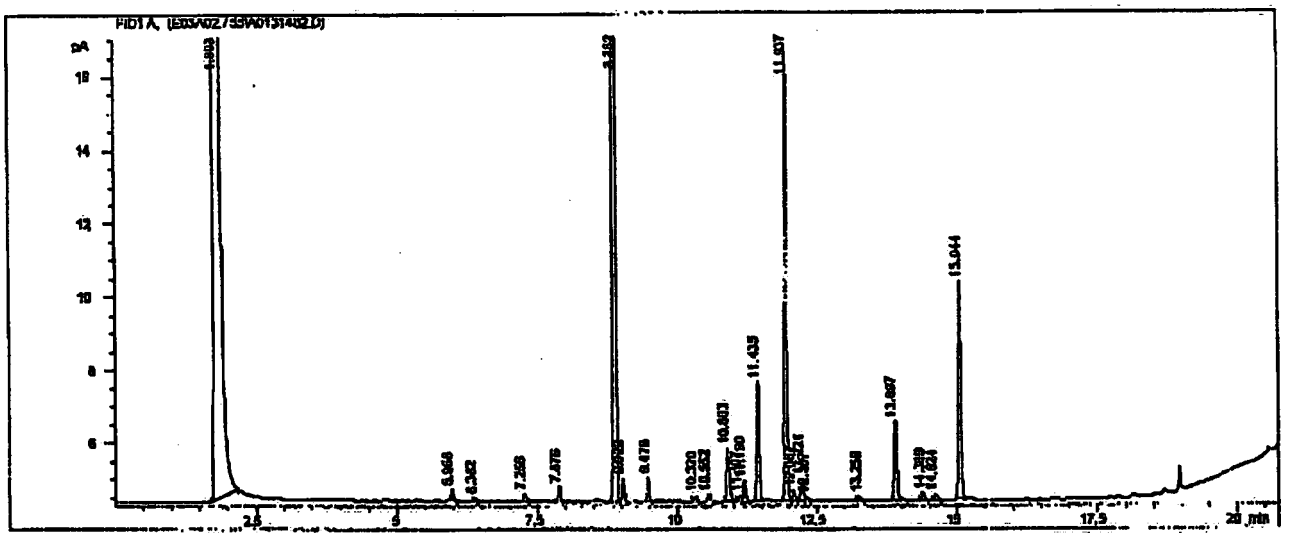
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.803	3.519E+8	0.028	----	7.004	SOLVENT PEAK	----	< min rt	
5.966	1455	0.037	1.048	12.613	13:0 ISO	0.33	ECL deviates -0.001	Reference -0.005
6.362	426	0.036	1.036	12.937	13:1 AT 12-13	0.09	ECL deviates 0.001	
7.256	1361	0.045	1.014	13.568	unknown 13.565	0.30	ECL deviates 0.003	
7.876	2155	0.040	1.000	13.998	14:0	0.46	ECL deviates -0.002	Reference -0.006
8.882	310905	0.040	0.981	14.627	15:0 ISO	65.57	ECL deviates 0.004	Reference 0.000
9.022	3393	0.042	0.979	14.714	15:0 ANTEISO	0.71	ECL deviates 0.001	Reference -0.003
9.478	3630	0.042	0.971	15.000	15:0	----	ECL deviates 0.000	
10.320	986	0.047	0.959	15.491	Sum In Feature 2	0.20	ECL deviates 0.003	14:0 3OH/16:1 ISO I
10.552	1055	0.041	0.956	15.626	16:0 ISO	0.22	ECL deviates -0.001	Reference -0.005
10.883	9842	0.049	0.951	15.819	Sum In Feature 3	2.01	ECL deviates -0.003	16:1 w7c/15 iso 2OH
11.107	979	0.048	----	15.950		----		
11.190	3115	0.044	0.947	15.998	16:0	0.63	ECL deviates -0.002	Reference -0.006
11.435	18907	0.045	0.945	16.136	15:0 ISO 3OH	3.84	ECL deviates 0.002	
11.937	70194	0.044	0.939	16.420	ISO 17:1 w9c	14.16	ECL deviates 0.004	
12.067	2093	0.054	0.937	16.493	Sum In Feature 4	0.42	ECL deviates 0.007	17:1 ANTEISO B/I I
12.221	3924	0.050	0.935	16.580	unknown 16.582	0.79	ECL deviates -0.002	
12.307	681	0.039	0.935	16.628	17:0 ISO	0.14	ECL deviates -0.002	Reference -0.005
13.256	826	0.041	0.925	17.162	16:0 ISO 3OH	0.16	ECL deviates 0.012	
13.897	13269	0.049	0.919	17.520	16:0 3OH	2.62	ECL deviates 0.001	
14.389	1161	0.045	----	17.795		----		
14.624	1059	0.050	0.912	17.926	18:1 w5c	0.21	ECL deviates 0.007	
15.044	36496	0.048	0.908	18.162	17:0 ISO 3OH	7.13	ECL deviates 0.001	Reference 0.000
----	986	----	----	----	Summed Feature 2	0.20	12:0 ALDE ?	unknown 10.928
----	----	----	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	9842	----	----	----	Summed Feature 3	2.01	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1 w7c
----	2093	----	----	----	Summed Feature 4	0.42	17:1 ISO I/ANTEI B	17:1 ANTEISO B/I I

ECL Deviation: 0.004 Reference ECL Shift: 0.004 Number Reference Peaks: 8
Total Response: 484282 Total Named: 482142
Percent Named: 99.56% Total Amount: 468822

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.301	Myroides-odoratus (Flavobacterium odoratum)

E03A027.55A [1482] 4632-2, 6



E03A027.55A [1483] 4632-2, 8 Page 1

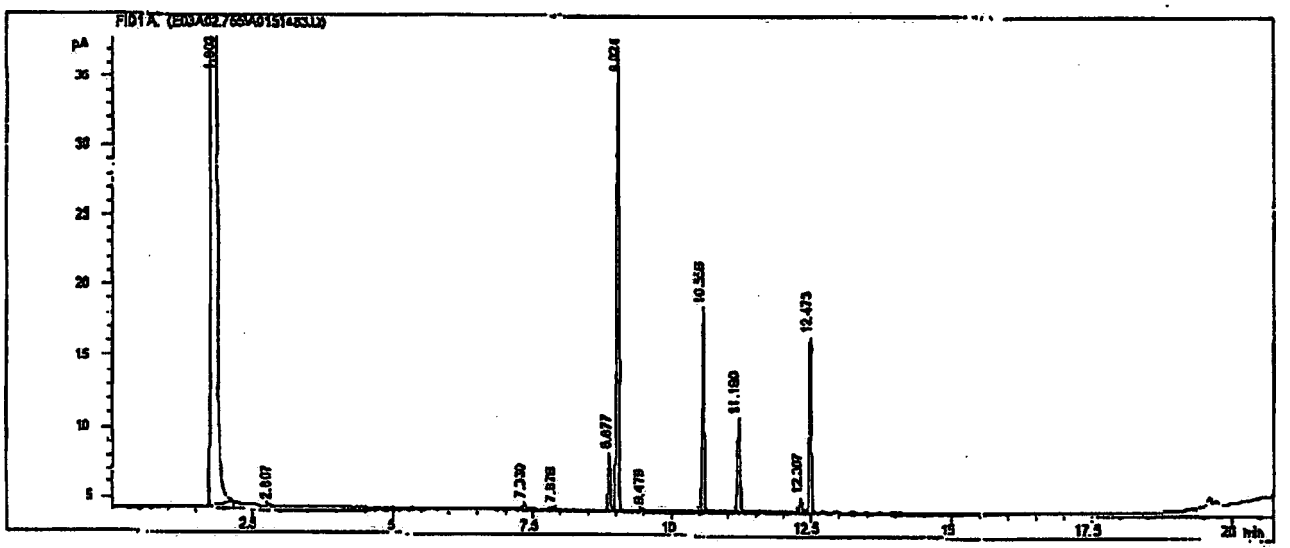
Volume: DATA File: E03A027.55A Seq Counter: 15 ID Number: 1483
Type: Samp Bottle: 13 Method: TSBA50
Created: 10/2/03 11:55:53 PM
Sample ID: 4632-2, 8

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.803	3.484E+8	0.029	----	6.999	SOLVENT PEAK	----	< min rt	
2.807	221	0.025	----	8.811		----	< min rt	
7.330	2298	0.037	1.002	13.619	14:0 ISO	0.63	ECL deviates 0.000	Reference 0.000
7.876	1146	0.040	0.991	13.998	14:0	0.31	ECL deviates -0.002	Reference -0.001
8.877	21094	0.040	0.973	14.623	15:0 ISO	5.59	ECL deviates 0.000	Reference 0.001
9.024	170140	0.041	0.971	14.715	15:0 ANTEISO	44.98	ECL deviates 0.002	Reference 0.002
9.479	961	0.039	0.964	14.999	15:0	----	ECL deviates -0.001	
10.556	77836	0.043	0.951	15.627	16:0 ISO	20.14	ECL deviates 0.000	Reference 0.001
11.190	36529	0.044	0.944	15.998	16:0	9.38	ECL deviates -0.002	Reference -0.002
12.307	5383	0.045	0.933	16.630	17:0 ISO	1.37	ECL deviates 0.000	Reference 0.000
12.473	69421	0.043	0.931	16.724	17:0 ANTEISO	17.60	ECL deviates 0.001	Reference 0.000

ECL Deviation: 0.001 Reference ECL Shift: 0.001 Number Reference Peaks: 8
Total Response: 383846 Total Named: 383846
Percent Named: 100.00% Total Amount: 368260

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.402	Rathayibacter-tritici (Clavibacter tritici)
	0.400	Rothia-dentocariosa
	0.390	Nesterenkonia-halobia (Micrococcus halobius)
	0.339	Microbacterium-liquefaciens (Aureobacterium liquefaciens)
	0.339	Microbacterium-esteraromaticum
	0.326	Microbacterium-hominis
	0.304	Kocuria-kristinae
	0.294	Microbacterium-laevaniformans (Corynebacterium laevaniformans
	0.294	Microbacterium-flavescens (Aureobacterium, Arthrobacter)
	0.258	Microbacterium-lacticum-GC subgroup A



Sherlock Version 4.5 (0209B) Printed on 03-Oct-2003

E03A027.55A [1484] 4632-2, 9

Volume: DATA File: E03A027.55A Seq Counter: 16 ID Number: 1484
Type: Samp Bottle: 14 Method: TSBA50
Created: 10/3/03 12:20:43 AM
Sample ID: 4632-2, 9

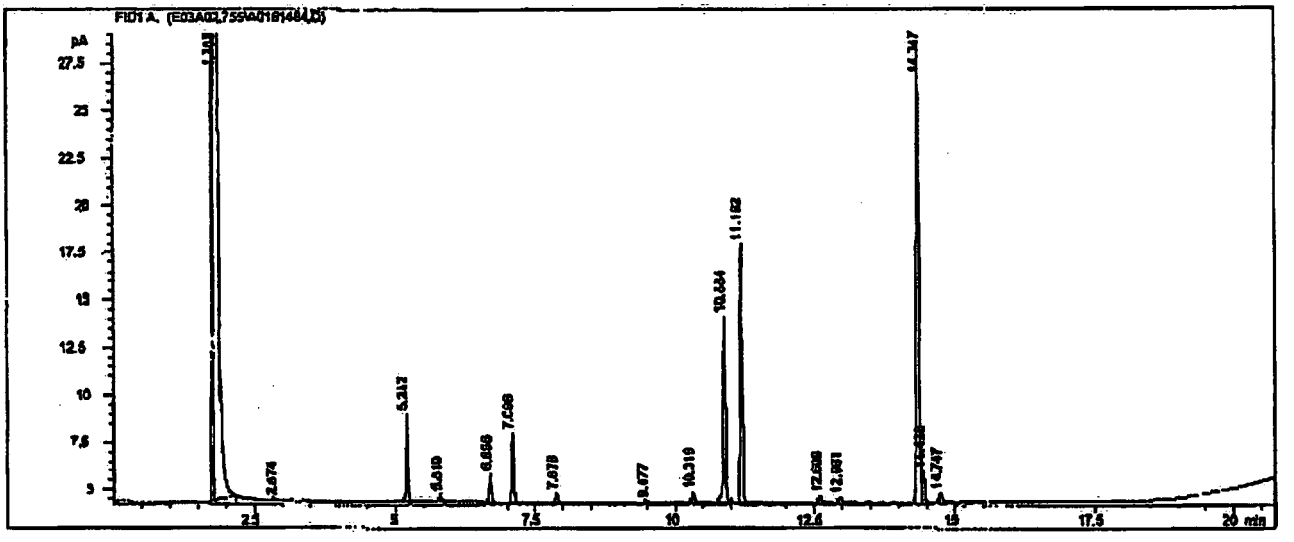
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.767	148678	0.017	—	6.937		—	< min rt	
1.803	3.51E+8	0.032	—	7.002	SOLVENT PEAK	—	< min rt	
2.874	235	0.026	—	8.936		—	< min rt	
5.212	19776	0.032	1.065	12.000	12:0	6.35	ECL deviates 0.000	Reference -0.002
5.810	1838	0.034	1.044	12.488	unknown 12.484	0.58	ECL deviates 0.004	
6.696	7161	0.036	1.017	13.180	12:0 2OH	2.19	ECL deviates 0.003	
7.096	17605	0.037	1.008	13.458	12:0 3OH	5.34	ECL deviates 0.004	
7.876	2519	0.039	0.991	14.000	14:0	0.75	ECL deviates 0.000	Reference -0.001
9.477	536	0.042	0.964	14.999	15:0	—	ECL deviates -0.001	
10.319	3041	0.045	0.953	15.490	Sum In Feature 2	0.87	ECL deviates 0.002	14:0 3OH/16:1 ISO I
10.884	57955	0.045	0.947	15.820	Sum In Feature 3	16.53	ECL deviates -0.002	16:1 w7c/15 iso 2OH
11.192	77093	0.044	0.944	16.000	16:0	21.91	ECL deviates 0.000	Reference -0.002
12.600	2281	0.046	0.930	16.797	17:1 w8c	0.64	ECL deviates 0.005	
12.961	1774	0.044	0.927	17.001	17:0	0.50	ECL deviates 0.001	Reference 0.000
14.347	147575	0.049	0.917	17.776	18:1 w9c	40.77	ECL deviates 0.007	
14.438	9701	0.046	0.917	17.826	18:1 w7c	2.68	ECL deviates 0.003	
14.747	3240	0.048	0.914	17.999	18:0	0.89	ECL deviates -0.001	Reference -0.003
—	3041	—	—	—	Summed Feature 2	0.87	12:0 ALDE ?	unknown 10.928
—	—	—	—	—		—	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
—	57955	—	—	—	Summed Feature 3	16.53	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.003 Reference ECL Shift: 0.002 Number Reference Peaks: 5
Total Response: 351561 Total Named: 351561
Percent Named: 100.00% Total Amount: 332524

Matches:

Library	Sim Index	Entry Name
TSBA50 5.00	0.817	Acinetobacter-radioresistens
	0.816	Acinetobacter-calcoaceticus
	0.610	Acinetobacter-baumannii
	0.568	Acinetobacter-haemolyticus
	0.506	Acinetobacter-genomospecies 3 (sensu IJSB 36:228-240)

E03A027.55A [1484] 4632-2, 9



Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:01
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: E. coli QC
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 42/103
Number +/b/- Reactions	: 42 / 4 / 50
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	<255>	20	5	10	<154>	<197>	11	<530>	6	6
B	2	<315>	<307>	<305>	5	<253>	8	<262>	<320>	<303>	<244>	<233>
C	<345>	{ 93}	<121>	4	<253>	<227>	8	<313>	8	-0	<224>	<131>
D	40	6	6	1	<237>	<273>	<277>	2	<314>	8	3	5
E	9	5	13	{ 86}	14	<323>	5	-0	8	12	1	<233>
F	<151>	1	19	{ 55}	{ 74}	<161>	<120>	<218>	<212>	12	<168>	4
G	4	5	5	1	0	14	1	<344>	<300>	<115>	1	4
H	4	<186>	<191>	<183>	4	0	11	2	<316>	<240>	<283>	<292>

=> Species ID: Escherichia coli <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Escherichia coli	100	0.87	1.88	GN-ENT
2)	Escherichia coli (USP5-7085)	0	0.00	5.06	GN-ENT
3)	Citrobacter freundii	0	0.00	6.77	GN-ENT
4)	Escherichia coli inactive	0	0.00	8.00	GN-ENT
5)	Citrobacter gillenii	0	0.00	8.11	GN-ENT
6)	Citrobacter amalonaticus	0	0.00	8.58	GN-ENT
7)	Escherichia coli O157:H7	0	0.00	8.70	GN-ENT
8)	Citrobacter braakii	0	0.00	8.75	GN-ENT
9)	Citrobacter sedlakii	0	0.00	8.80	GN-ENT
10)	Citrobacter werkmanii	0	0.00	9.15	GN-ENT
Other)					

Print Time = Oct 03 2003 16:01

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:02
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-1(1) Plate Type: GN2
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 78/159
Number +/b/- Reactions	: 47 / 7 / 42
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
(X): borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	<377>	(95)	-3	-4	<752>	<886>	1	<727>	-7	25+
B	-3	<481>	<385>	<705>	9+	<369>	<509>	<401>	1	<563>	<359>	<534>
C	<578>	<320>	(118)	<281>	<284>	<409>	<553>	<529>	13	-7	<472>	<283>
D	(123)	0	13	<260>	-5	<906>	<977>	<636>	<858>	(136)	-1	-8
E	-3	5	64	(92)	5	<620>	-3	<178>	-1	5	-8	<382>
F	<382>	-5	9	14	<217>	<359>	<345>	<406>	<373>	(126)	<396>	<227>
G	-4	-5	-8	4	4	1	5	<681>	<503>	5	-8	-6
H	-3	<432>	<542>	<250>	-5	(146)	1	-7	<478>	<414>	<716>	<758>

=> Species ID: Citrobacter freundii <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Citrobacter freundii	100	0.87	1.97	GN-ENT
2)	Escherichia coli	0	0.00	6.53	GN-ENT
3)	Citrobacter farmeri	0	0.00	6.93	GN-ENT
4)	Citrobacter braakii	0	0.00	7.37	GN-ENT
5)	Escherichia coli (USP5-7085)	0	0.00	7.61	GN-ENT
6)	Citrobacter sedlakii	0	0.00	8.18	GN-ENT
7)	Citrobacter koseri	0	0.00	8.92	GN-ENT
8)	Escherichia coli O157:H7	0	0.00	9.12	GN-ENT
9)	Raoultella planticola/ornithinolytica	0	0.00	9.14	GN-ENT
10)	Raoultella planticola	0	0.00	9.25	GN-ENT
Other)					

Print Time = Oct 03 2003 16:02 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:03
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-1(3) Plate Type: GN2
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic Color: 44/142
Number +/b/- Reactions	: 35 / 8 / 53
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	5	<384>	18	5	2	1	<759>	2	<955>	3+	<337>
B	3	<519>	2	<668>	<292>	<548>	<397>	16	4	<577>	<559>	<625>
C	4	<911>	{117}	11	<445>	25	<706>	<1014>	21	3	<216>	{130}
D	14	<567>	<508>	26	<474>	<228>	<717>	-1	<211>	5	-3	2
E	0	5	20	15	6	<168>	4	14	25	<199>	-8	<279>
F	<291>	1	13	3	5	<156>	12	<314>	{83}	<184>	4	7
G	3	-1	-4	-4	-7	{115}	{89}	7	{101}	-4	-2	{73}
H	-7	<404>	29	{125}	3	0	-3	-2	<485>	<208>	<757>	<528>

=> Species ID: Pantoea dispersa <=

Species	PROB	SIM	DIST	TYPE
=>1) Pantoea dispersa	100	0.86	2.14	GN-ENT
2) Pantoea agglomerans	0	0.00	4.52	GN-ENT
3) Enterobacter gergoviae	0	0.00	7.57	GN-ENT
4) Enterobacter agglomerans bgp 7 (Pantoea)	0	0.00	8.40	GN-ENT
5) Enterobacter amnigenus	0	0.00	8.62	GN-ENT
6) Enterobacter agglomerans bgp 4 (Pantoea)	0	0.00	8.66	GN-ENT
7) Enterobacter cancerogenus	0	0.00	9.21	GN-ENT
8) Pectobacterium cypripedii	0	0.00	9.30	GN-ENT
9) Enterobacter asburiae	0	0.00	9.32	GN-ENT
10) Enterobacter cloacae	0	0.00	9.34	GN-ENT
Other)				

Print Time = Oct 03 2003 16:03

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\csh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:04
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-1(7) Plate Type: GN2
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 98/129
Number +/b/- Reactions	: 41 / 4 / 51
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
(X): borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	2	<187>	21	6	12	4+	<557>	1	<546>	7	<241>
B	5	<310>	5	<328>	<215>	<416>	5	<144>	<150-	<281>	<288>	<338>
C	<288>	<394>	<233>	<278>	<164>	<236>	<446>	<427>	(126)	3	<268>	(110)
D	4+	0	<291>	89	0	<319>	<505>	4	<271>	6	-2	-3
E	<150>	1	7	6	12	<355>	4	5	77	9	-3	<150>
F	<202>	-1	59	5	(109)	<241>	<179>	12+	<257>	<149>	7+	7+
G	0	4	-1	3	7	4	0	<522>	<311>	7	-1	0
H	-1	<285>	<181>	(118)	3	6	0	-3	<288>	1	<457>	<476>

=> Species ID: Kluyvera ascorbata <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Kluyvera ascorbata	97	0.59	6.07	GN-ENT
2)	Enterobacter cloacae	2	0.01	7.36	GN-ENT
3)	Rahnella aquatilis	0	0.00	7.92	GN-ENT
4)	Enterobacter intermedius	0	0.00	8.38	GN-ENT
5)	Enterobacter amnigenus	0	0.00	8.43	GN-ENT
6)	Citrobacter gillenii	0	0.00	9.02	GN-ENT
7)	Enterobacter asburiae	0	0.00	9.03	GN-ENT
8)	Kluyvera cryocrescens	0	0.00	9.09	GN-ENT
9)	Raoultella planticola/ornithinolytica	0	0.00	9.09	GN-ENT
10)	Enterobacter nimipressuralis	0	0.00	9.60	GN-ENT
Other)					

Print Time = Oct 03 2003 16:04

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:05
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-1(6) (clumpy soln)
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 50/97
Number +/b/- Reactions	: 14 / 5 / 77
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	8	< 474>	17	9	17	-2	-0+	-2	{ 73}	2	< 431>
B	-0	< 488>	9	< 351>	< 215>	< 443>	-2	< 345-	11	< 399>	-4	< 434>
C	2	34+	{ 82}	5	9	23	< 449-	< 239>	22	7	{ 58}	-5
D	9	2	1	3	2	3	< 227>	2	2	24	9	3
E	< 210-	1	21	7	10	14	-3	{ 75}	5	1	-1	3
F	3	0	33	45	8	10	18	9	8	7	8	7
G	14	7	2	8	3	8	3	11	-2	7	32	10
H	1	{ 66}	14	50	1	< 166-	-3	-5	42	2	2+	-7+

=> No ID <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Kluyvera cryocrescens	---	0.36	7.99	GN-ENT
2)	Obesumbacterium proteus biogroup 2	---	0.05	8.63	GN-ENT
3)	Leclercia adecarboxylata	---	0.02	8.95	GN-ENT
4)	Escherichia vulneris	---	0.01	9.05	GN-ENT
5)	Pantoea dispersa	---	0.01	9.12	GN-ENT
6)	Buttiauxella noackiae	---	0.01	9.14	GN-ENT
7)	Buttiauxella warmboldiae	---	0.01	9.15	GN-ENT
8)	Enterobacter intermedius	---	0.01	9.40	GN-ENT
9)	Rahnella aquatilis	---	0.00	9.46	GN-ENT
10)	Cedecea davisae	---	0.00	9.78	GN-ENT
Other)					

Print Time = Oct 03 2003 16:05

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:07
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-2 (1) Plate Type: GN2
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 98/149
Number +/b/- Reactions	: 39 / 3 / 54
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN501.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
(X): borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	2	<283>	9	6	9	<214-	<451>	4	<755>	3	<220>
B	7	<251>	7	<214>	<192>	<287>	5	<238>	80	<272>	<254>	<242>
C	<192>	<269>	36+	<219>	<170>	6+	<270>	<293>	{133}	0	<172>	13
D	57	2	<318>	{115}	3	<261>	<359>	14	<204>	1	1	0
E	5	3	6	5	8	<318>	0	7	33	4	-4	{104}
F	<206>	1	22	8	7+	<185>	<184>	12+	<221-	35	<222>	<168>
G	1	7	1	10	2	-2	1	<516>	<416>	9	1	1
H	1	<293>	<209>	<153>	-1	-1	1	-5	<359>	2	<385>	<393>

=> No ID <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Enterobacter intermedius	---	0.40	6.08	GN-ENT
2)	Kluyvera cryocrescens	---	0.11	6.49	GN-ENT
3)	Kluyvera ascorbata	---	0.08	6.62	GN-ENT
4)	Kluyvera georgiana	---	0.01	7.29	GN-ENT
5)	Enterobacter amnigenus	---	0.00	8.12	GN-ENT
6)	Buttiauxella agrestis	---	0.00	8.49	GN-ENT
7)	Kluyvera cochlease	---	0.00	8.88	GN-ENT
8)	Raoultella terrigena	---	0.00	9.28	GN-ENT
9)	Enterobacter nimipressuralis	---	0.00	9.38	GN-ENT
10)	Escherichia vulneris	---	0.00	9.82	GN-ENT
Other)					

Print Time = Oct 03 2003 16:07

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:08
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-2 (2) Plate Type: GN2
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 98/154
Number +/b/- Reactions	: 41 / 8 / 49
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	<255>	68	4	13	<209>	<747>	2	<1099>	0	<209>
B	1	<340>	4	<410>	<208>	<306>	<284>	<337>	7	<413>	<345>	<302>
C	<363>	<411>	{131}	<376>	0	<447>	<322>	<448>	{116}	-4	<334>	8
D	{106}	<263>	<402>	{132}	-1+	<449>	<757>	3	<376>	2	-1	-1
E	<395>	8	9	4	6	<410>	4	2	8	5	-4	29+
F	<197>	-2	1	8	11+	<323>	<166>	<277>	<225>	<172>	10	67+
G	1	3	1	7	5	7	-3	<623>	<339>	3	-3	-1
H	1	<384>	<214>	{136}	-2	-1	1	-4	<707>	{139}	<445>	<536>

=> Species ID: Enterobacter cloacae <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Enterobacter cloacae	100	0.89	4.76	GN-ENT
2)	Kluyvera ascorbata	0	0.00	7.27	GN-ENT
3)	Raoultella terrigena	0	0.00	7.79	GN-ENT
4)	Enterobacter amnigenus	0	0.00	7.88	GN-ENT
5)	Serratia plymuthica	0	0.00	7.93	GN-ENT
6)	Enterobacter asburiae	0	0.00	7.94	GN-ENT
7)	Raoultella planticola/ornithinolytica	0	0.00	8.14	GN-ENT
8)	Klebsiella pneumoniae ss pneumoniae	0	0.00	8.41	GN-ENT
9)	Enterobacter nimipressuralis	0	0.00	8.88	GN-ENT
10)	Kluyvera georgiana	0	0.00	9.33	GN-ENT
Other)					

Print Time = Oct 03 2003 16:08

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:09
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-2 (4)
Strain Type	: GN-ENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 63/87
Number +/b/- Reactions	: 38 / 2 / 58
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	4	<457>	21	-9	-8	-3	<942>	1	<781>	<884>	<597>
B	7	<548>	20	<818>	<220>	<541>	<883>	<651-	35	<450>	<904>	<1080>
C	<760-	<575>	50+	<734-	<570>	<825-	<736>	<690>	42	-2	<128>	<135>
D	18	<250>	<213>	-3	1+	29	<957>	<229>	28	25	1	-1
E	7	-1	51	42	4	<270>	-0	13	{80}	19	-4	<274>
F	<377>	15	2	18	18	<108>	17	<105>	22+	25	7	17
G	12	-6	5	1	-8	30	9	13	14+	-2	-1	-4
H	-4	{73}	60	<236>	8	-6	10	-4	<1047>	48+	<895>	<694>

=> No ID <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Pantoea dispersa	---	0.40	9.93	GN-ENT
2)	Pantoea stewartii ss stewartii	---	0.00	13.25	GN-ENT
3)	Raoultella planticola/ornithinolytica	---	0.00	13.35	GN-ENT
4)	Raoultella terrigena	---	0.00	13.36	GN-ENT
5)	Serratia rubidaea	---	0.00	13.68	GN-ENT
6)	Kluyvera cryocrescens	---	0.00	13.94	GN-ENT
7)	Enterobacter intermedius	---	0.00	14.01	GN-ENT
8)	Enterobacter agglomerans bgp 6 (Pectobacterium)	---	0.00	14.16	GN-ENT
9)	Enterobacter cloacae	---	0.00	14.16	GN-ENT
10)	Enterobacter sakazakii	---	0.00	14.21	GN-ENT
Other)					

Print Time = Oct 03 2003 16:09

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\ceh100303.D4C
Unrestricted Access?	: Yes
Read Time	: Oct 03 2003 16:10
Parent File	: Original Data Record
Plate Number	: 1
Incubation Time	: 16-24
Sample Number	: 4632-2 (9)
Strain Type	: GN-NENT
Strain Number	:
Strain Name	:
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 75/115
Number +/b/- Reactions	: 27 / 7 / 62
Database To Search	: MicroLog
Data Base(s) Searched	: C:\Biolog420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
(X): borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	55	24	< 134>	< 509>	< 500>	24	24	5	(111)	-51	-41
B	-20	18	< 707>	12	30	25	1	27	-57	30	18	9
C	22	0	(81)	6	15	46	12	56	42	40	< 541>	< 518>
D	< 148>	75	< 617>	-13	9	56	40	-24	-6	< 159>	< 558>	< 706>
E	-33	46	(100)	< 182>	-11	< 626>	-54	(96)	< 539>	< 536-	< 486>	< 179>
F	< 334>	(92)	18	< 253>	< 659>	< 633>	60	63+	55+	< 395>	49	25
G	(83)	19	-46	-36	-13	< 557>	< 781>	13	< 154-	82	< 205>	< 765>
H	(82)	4	3	18	-6	21	-10	10+	-55	-6	41	6

=> Species ID: Acinetobacter genospecies 10 <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Acinetobacter genospecies 10	100	0.65	5.45	GN-NENT OXI-
2)	Acinetobacter genospecies 11	0	0.00	8.51	GN-NENT OXI-
3)	Acinetobacter junii/genospecies 5	0	0.00	10.30	GN-NENT OXI-
4)	Pseudomonas oleovorans	0	0.00	11.14	GN-NENT OXI+
5)	Pseudomonas pseudoalcaligenes ss pseudoalcaligenes	0	0.00	12.43	GN-NENT OXI+
6)	Acinetobacter johnsonii/genospecies 7	0	0.00	12.52	GN-NENT OXI-
7)	Acinetobacter calcoaceticus/genospecies 3	0	0.00	13.57	GN-NENT OXI-
8)	Acinetobacter calcoaceticus/genospecies 1	0	0.00	13.66	GN-NENT OXI-
9)	Pseudomonas mendocina	0	0.00	13.70	GN-NENT OXI+
10)	Ralstonia solanacearum	0	0.00	13.98	GN-NENT OXI+
Other)					

Print Time = Oct 03 2003 16:10

Appendix D

Data Quality Review

2690 Oak Hill Dr.
Allison Park, PA 15101
Phone: 412-486-6989

Environmental Data Services

To: Cliff Firstenberg

Mark Harris

From: Diane Waldschmidt

Date: 4/26/04

Re: Data Review of Water Born Pathogen Analyses

Samples reviewed as listed on field COC for Giardia and Cryptosporidium analyses:

CSO01-SAY-092303 (tag number 63147)
CSO01-SAY-092303 (tag number 63161)

Giardia: USEPA Method 1623

Samples were received by the laboratory at the required temperature.

Samples appear to be processed and analyzed within method holding time requirements.

Recovery efficiency of the known control (ColorSeed) associated with each of the samples evaluated are lower than the laboratory's established control criteria (15-118%). It should be noted that raw bench notes were not identified and are therefore not available to verify the authenticity of the ColorSeed recoveries reported at the time of this evaluation.

The Method blank processed in association with the samples evaluated was negative for Giardia.

An OPR control was processed along with the samples evaluated. Estimated recovery of the OPR control standard is 72%.

Sample results reported were verified through examination of lab bench notes.

Cryptosporidium: USEPA Method 1623

Samples were received by the laboratory at the required temperature.

Samples appear to be processed and analyzed within method holding time requirements.

Recovery efficiency of the known control (ColorSeed) associated with sample CSO01-SAY-092303 (tag 63161) is lower than the laboratory's established control criteria (13-143%). Sample CSO01-SAY-092303 (tag 63147) has a recovery efficiency of 16.2%. Although this recovery falls within the window of acceptance, it is rather low. It should be noted that raw bench notes were not identified and are therefore not available to verify the authenticity of the ColorSeed recoveries reported at the time of this evaluation.

The Method blank processed in association with the samples evaluated was negative for Cryptosporidium.

An OPR control was processed along with the samples evaluated. Estimated recovery of the OPR control standard is 46%.

Sample results reported were verified through examination of lab bench notes.

Samples reviewed as listed on field COC for SM 9222B, 9222 D, 9222G, 9230C and Bacterial Identification by Agar Culture analyses:

CSO01-SAY-092303 (tag number 63147)
CSO02-SAY-092303 (tag number 63153)

The laboratory did not provide documentation or discussion relative to sample condition upon receipt. It is assumed that samples were received in good condition and at the appropriate temperature, because the samples outlined above were received by the lab on the same day they were collected, and no indication is given to the contrary.

Documentation provided indicates that the samples were prepared and analyzed within the method stipulated holding times.

No documentation was provided indicating that a method blank was processed in association with the field samples listed above. Therefore no evaluation of potential artificial contamination could be performed.

Sample results reported were verified through examination of laboratory bench notes. Numerical results and positive identifications reported for methods SM9222B, 9222D, 9222G, and 9230C have been verified. Only the identification of the bacteria Agar Cultures could be verified based on the documentation provided. The numerical values were not checked due to lack of information.

The following laboratory narration should be considered when using the bacterial identification results in the cases listed below. "Not all organisms were identified adequately, which is why some bacteria were listed as "resembling" a certain organism, the closest match we could make".

CSO02-SAY-092303

Gram Negative Rods resembling Enterbacter species
Gram Variable Rods resembling Myroides odoratus
Gram Negative Rods resembling Pantoea species